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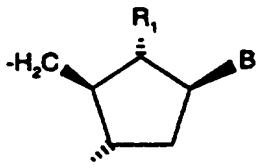
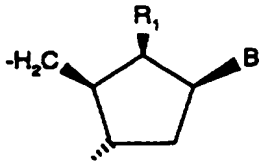
(54) Title: 6'-SUBSTITUTED CARBOCYCLIC NUCLEOSIDES

(57) Abstract

Oligonucleotides which comprise from 2 to 200 identical or different residues of natural or synthetic nucleosides, which are linked via a nucleotide-bridging group Y, in which at least two of the nucleosides are, independently of each other, a residue of formula (Ia or Ib), in which R_1 is OH or NH_2, and B is a pyrimidine or purine residue or an analogue thereof, and at least two of these nucleosides are consecutive on at least one occasion.



(Ia)



(Ib)

Applicants: Arlindo L. Castelhano, et al.
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6'-Substituted carbocyclic nucleosides

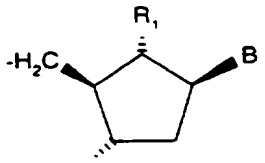
The invention relates to oligonucleotides possessing at least two consecutive 6'-hydroxyl-substituted or 6'-amino-substituted carbocyclic nucleotide units, to 6'-amino-substituted carbocyclic nucleoside analogues, to a process for preparing the oligonucleotides and to their use.

Nucleosides and oligonucleotides have attracted wide interest as compounds with anti-viral activity or because of their ability to interact with nucleic acids ("antisense" oligonucleotides) and the biological activity associated therewith. In order to provide nucleosides possessing novel properties, or to improve the interaction of antisense oligonucleotides with natural nucleic acids, and also to improve their stability towards nucleases, the sugar residues of nucleosides (or of the nucleotide units in oligonucleotides), or the internucleotide phosphate bond in oligonucleotides, have been modified in a very wide variety of ways, cf., for example, Milligan, J.F., Matteucci, M.D., Martin, J.C., J. Med. Chem. 36:1923-1947 (1993). In this connection, oligonucleotides which contain carbocyclic nucleotide units, in which the oxygen atom of the furanose ring is therefore replaced by a CH_2 group, have already been previously described in the literature, cf., for example, Moser, H. E., Perspectives Medicinal Chemistry 275-297 (1993). In this context, the carbon atom which replaces the ring oxygen is generally designated C-6'. Various derivatives of 6'-hydroxyl-substituted carbocyclic nucleosides have been described as nucleoside analogues which possess antiviral activity, for example in Borthwick, A.D., Biggadike, K., Tetrahedron 48:571-623 (1992). Oligonucleotides are also known which contain a 6'-hydroxyl-substituted carbocyclic nucleoside (WO 91/10671).

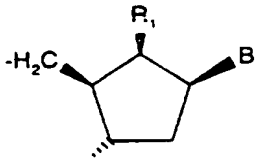
It has now been found that oligonucleotides which contain at least two consecutive nucleotide units selected from the group consisting of 6'-hydroxyl-substituted and 6'-amino-substituted carbocyclic nucleotides display elevated biological activity. These oligonucleotides bind very well to the target RNA and are also very resistant towards degradation by nucleases.

An object of the present application are oligonucleotides which contain from 2 to 200 identical or different residues of natural or synthetic nucleosides which are linked via a

nucleotide-bridging group Y, wherein at least two of the nucleosides are, independently of each other, a residue of the formula Ia or Ib



(Ia)

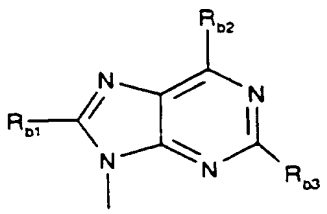


(Ib)

in which R_1 is OH or NH_2 and B is a pyrimidine or purine residue, or an analogue thereof, and at least two of these nucleosides are consecutive on at least one occasion.

For example, three or more of the nucleosides in the oligonucleotides according to the present application can, independently of each other, be a residue of the formula Ia or Ib; furthermore, three or more of these nucleosides can, for example, be consecutive.

If B is a purine residue or an analogue thereof, this can then be a residue of the formula II, IIa, IIb, IIc, IId, IIe or IIf,



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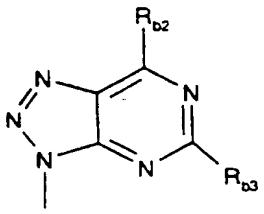

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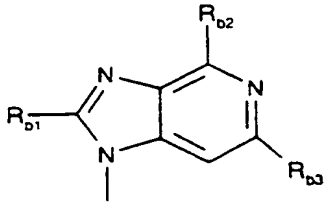

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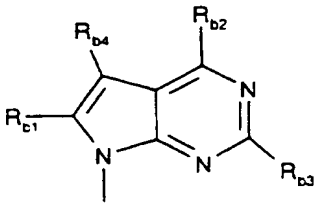

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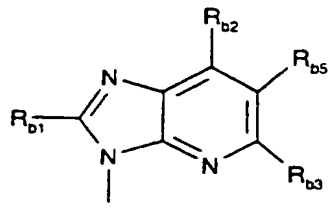
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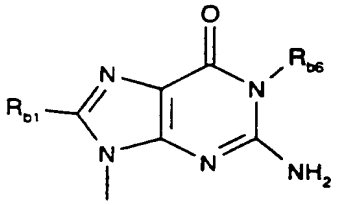
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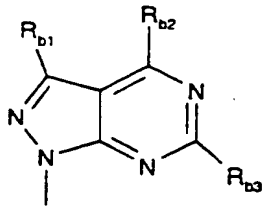

(IIf),

in which R_{b1} is H, Cl, Br, OH or -O-C_1-C_{12}alkyl, R_{b2}, R_{b3} and R_{b5} are, independently of each other, H, OH, SH, NH_2, $NHNH_2$, NHOH, NHO-C_1-C_{12}alkyl, -N=CH-N(C_1-C_{12}alkyl)$_2$, -N=CH-N-cycloalkyl, F, Cl, Br, C_1-C_{12}alkyl, hydroxy-C_1-C_{12}alkyl, amino-C_1-C_{12}alkyl, C_1-C_{12}alkoxy, benzyloxy, C_1-C_{12}alkylthio, where the hydroxyl and amino groups are unsubstituted or substituted by a protecting group, phenyl, benzyl, primary amino having from 1 to 20 C atoms, or secondary amino having from 2 to 30 C atoms, R_{b4} is hydrogen, CN or -C≡C-R_{b7}, and R_{b6} and R_{b7} are hydrogen or C_1-C_4alkyl.

Suitable protecting groups are those which are well known in nucleotide chemistry. Preferred protecting groups are C_1-C_8acyl groups, for example acetyl, propionyl, butyryl and benzoyl. R_{b6} is preferably H or methyl.

The primary amino preferably contains from 1 to 12, and particularly preferably from 1 to 6, C atoms, and the secondary amino preferably contains from 2 to 12, and particularly preferably from 2 to 6, C atoms.

Some examples of alkyl, alkoxy, alkylthio, hydroxyalkyl and aminoalkyl, which preferably contain from 1 to 6 C atoms, are methyl, ethyl and the isomers of propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, undecyl and dodecyl, and also corresponding alkoxy, alkylthio, hydroxyalkyl and aminoalkyl radicals. The alkyl, alkoxy, alkylthio, hydroxyalkyl and aminoalkyl particularly preferably contain from 1 to 4 C atoms. Preferred alkyl, alkoxy,

alkylthio, hydroxyalkyl and aminoalkyl radicals are methyl, thyl, n- and i-propyl, n-, i- and t-butyl, methoxy, ethoxy, methylthio and ethylthio, aminomethyl, aminoethyl, hydroxymethyl and hydroxyethyl.

The primary amino and secondary amino radicals can, for example, be radicals of the formula $R_{a1}R_{a2}N$, in which R_{a1} is H or, independently, has the meaning of R_{a2}, and R_{a2} is C_1-C_{20}-, preferably C_1-C_{12}- and, particularly preferably, C_1-C_6-alkyl, -aminoalkyl or -hydroxyalkyl; carboxyalkyl or carbalkoxyalkyl, where the carbalkoxy group contains from 2 to 8 C atoms and the alkyl group contains from 1 to 6, preferably from 1 to 4 C atoms; C_2-C_{20}-, preferably C_2-C_{12}- and, particularly preferably C_2-C_6-alkenyl; phenyl, mono- or di-(C_1-C_4-alkyl- or -alkoxy)phenyl, benzyl, mono- or di-(C_1-C_4-alkyl- or -alkoxy)benzyl; or 1,2-, 1,3- or 1,4-imidazolyl-C_1-C_6alkyl, or R_{a1} and R_{a2} are, together, tetra- or penta-methylene, 3-oxa-1,5-pentylene, $-CH_2-NR_{a3}-CH_2CH_2-$ or $-CH_2CH_2-NR_{a3}-CH_2CH_2-$, in which R_{a3} is H or C_1-C_4alkyl. The amino group in the aminoalkyl can be substituted by one or two C_1-C_4alkyl or C_1-C_4hydroxyalkyl groups. The hydroxyl group in the hydroxyalkyl can be etherified with C_1-C_4alkyl.

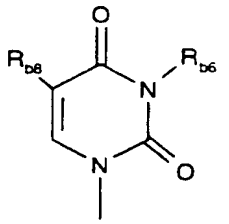
Examples of alkyl have been given previously. Examples of aminoalkyl are aminomethyl, aminoethyl, 1-aminoprop-2-yl or -3-yl, 1-amino-but-2-yl or -3-yl or -4-yl, N-methyl- or N,N-dimethyl- or N-ethyl- or N,N-diethyl- or N-2-hydroxyethyl- or N,N-di-2-hydroxyethylaminomethyl or -aminoethyl or -aminopropyl or -aminobutyl. Examples of hydroxyalkyl are hydroxymethyl, 1-hydroxyeth-2-yl, 1-hydroxyprop-2- or -3-yl, 1-hydroxybut-2-yl, -3-yl or -4-yl. Examples of carboxyalkyl are carboxymethyl, carboxyethyl, carboxypropyl and carboxybutyl, and examples of carbalkoxyalkyl are these carboxyalkyl groups which are esterified with methyl or ethyl. Examples of alkenyl are allyl, but-1-en-3-yl or -4-yl, pent-3- or 4-en-1-yl or -2-yl, hex-3- or -4- or -5-en-1-yl or -2-yl. Examples of alkyl- and alkoxyphenyl or -benzyl are methylphenyl, dimethylphenyl, ethylphenyl, diethylphenyl, methylbenzyl, dimethylbenzyl, ethylbenzyl, diethylbenzyl, methoxyphenyl, dimethoxyphenyl, ethoxyphenyl, diethoxyphenyl, methoxybenzyl, dimethoxybenzyl, ethoxybenzyl or diethoxybenzyl. Examples of imidazolylalkyl, in which the alkyl group preferably contains from 2 to 4 C atoms, are 1,2-, 1,3- or 1,4-imidazolylethyl or -n-propyl or -n-butyl. R_{a3} is preferably H, methyl or ethyl.

Preferred examples of primary amino and secondary amino are methyl-, ethyl-, dimethyl-, diethyl-, allyl-, mono- or di-(1-hydroxyeth-2-yl)-, phenyl- and benzylamino, acetylamino, isobutyrylamino and benzoylamino.

In a preferred embodiment, R_{b1} is hydrogen. In another preferred embodiment, R_{b5} is hydrogen. In a further preferred embodiment, R_{b2} and R_{b3} are, independently of each other, H, F, Cl, Br, OH, SH, NH_2, NHOH, $NHNH_2$, methylamino, dimethylamino, benzoylamino, isobutyrylamino, -N=CH-N(C_1-C_{12}alkyl)$_2$, -N=CH-N-cycloalkyl, methoxy, ethoxy and methylthio.

Some examples of analogues of the purine series, besides purine itself, are xanthine, hypoxanthine, adenine, N-methyladenine, N-benzoyladenine, 2-methylthioadenine, 2-aminoadenine, 6-hydroxypurine, 2-amino-6-chloropurine, 2-amino-6-methylthiopurine, guanine and N-isobutyrylguanine. Adenine, 2-aminoadenine and guanine are particularly preferred, as are their base-protected derivatives.

If B in formula Ia or Ib is a pyrimidine residue or an analogue thereof, this is then preferably a uracil, thymine or cytosine residue of the formula III, IIIa, IIIb or IIIc



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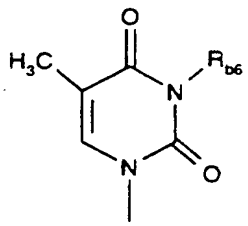

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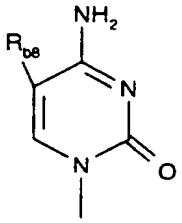

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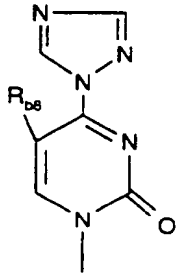


(IIIc)

in which R_{b6} is H or C_1-C_4alkyl and R_{b8} is H, OH, SH, NH_2, $NHNH_2$, NHOH, NHO-C_1-C_{12}-alkyl, -N=CH-N(C_1-C_{12}alkyl)$_2$, -N=CH-N-cycloalkyl, F, Cl, Br, C_1-C_{12}alkyl, hydroxy-C_1-C_{12}-alkyl, amino-C_1-C_{12}alkyl, C_1-C_{12}alkoxy, benzyloxy, C_1-C_{12}alkylthio, where the hydroxyl and amino groups are unsubstituted or substituted by a protecting group, phenyl, benzyl, primary amino having from 1 to 20 C atoms, secondary amino having from 2 to 30 C atoms, C_1-C_{12}-alkenyl or C_1-C_{12}alkynyl, and the NH_2 group in formula IIIb is unsubstituted or substituted by C_1-C_6alkyl, benzoyl or a protecting group, and also the dihydro derivatives of the residues of the formulae III, IIIa, IIIb and IIIc. Preferably, R_{b8} in formula III is H, C_1-C_6-alkyl or -hydroxyalkyl, C_2-C_6-alkenyl or -alkynyl, F, Cl, Br, NH_2, benzoylamino or mono- or di-C_1-C_6-alkylamino. Preferably, R_{b8} in formula IIIb and IIIc is H, C_1-C_6-alkyl or -alkoxy or -hydroxyalkyl, C_2-C_6-alkenyl or -alkynyl, F, Cl, Br, NH_2, benzoylamino or mono- or di-C_1-C_6alkylamino.

R_{b6} is preferably H or methyl. R_{b8} in formula III is preferably H, F, Cl, Br, NH_2, $NHCH_3$, $N(CH_3)_2$, C_1-C_4alkyl, C_2-C_4alkenyl or C_2-C_4alkyn-1-yl. R_{b8} in formula IIIb and IIIc is preferably H, C_1-C_4alkyl, particularly methyl, C_2-C_4alkenyl, particularly vinyl or C_2-C_4alkyn-1-yl, particularly 1-propyn-1-yl, or NH_2, $NHCH_3$ or $(CH_3)_2N$.

Some examples of pyrimidine analogues are uracil, thymine, cytosine, 5-fluorouracil, 5-chlorouracil, 5-bromouracil, dihydrouracil, 5-methylcytosine, 5-propynyluracil and 5-propynylcytosine.

Preferred bridging groups Y are the group -P(O)O^--, which occurs in natural oligonucleotides, and also -P(O)S^--. Thus an oligonucleotide according to the present invention can be completely composed of the bridging group -P(O)O^--, or it can be completely composed of the bridging group -P(O)S^--. In a further preferred embodiment the bridging group Y within the same oligonucleotide at each position is independently -P(O)O^-- or -P(O)S^--. Examples of additional bridging groups are -P(S)S^--, -P(O)R_2-, P(O)NR_3R_4, or -CH_2-, in which R_2 is H or C_1-C_6alkyl, and R_3 and R_4, independently of each other, have the meaning of R_2.

The oligonucleotides according to the present invention preferably contain from 2 to 100, particularly preferably from 3 to 50 and, especially preferably, from 5 to 29, nucleoside

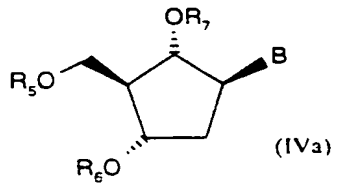
residues. The choice and the order of the building blocks in the sequence of the oligonucleotide are determined by the necessity of forming a duplex with a target RNA. In this context, the oligonucleotide can be constructed partially or completely from natural DNA building blocks which are complementary to a partial sequence of the target RNA or be constructed completely or partially from unnatural synthetic nucleotides which are likewise complementary to a partial sequence of the target RNA, with partial meaning that natural DNA building blocks which are complementary to the target RNA are replaced in the oligonucleotide sequence with unnatural synthetic nucleotides which are likewise complementary.

The residues of the formulae Ia and/or Ib can be bonded terminally or within the nucleotide sequence, with all or several, at least, however, 2, of the residues of the formulae Ia and/or Ib being consecutive. Depending on the length of the oligonucleotide, from 2 to all, preferably from 2 to 50, more preferably from 2 to 30, particularly preferably from 3 to 20 and, especially preferably, from 4 to 10, nucleotides can be residues which are selected from the group consisting of residues of the formula Ia and Ib, which are either all consecutive or else distributed over the sequence, with it being necessary for 2 such residues to be consecutive on at least one occasion. Thus, there can be a contiguous region which is present at the 5' or 3' end or somewhere within the sequence, the residues can be distributed randomly over the sequence, with 2 such residues being consecutive on at least one occasion, or there can be several, for example 2, 3, 4 or more, contiguous regions which are of equal length or, independently of each other, of differing lengths, and which are distributed over the sequence. According to the invention, 2 such residues, or, for example, 3, 4, 5, 6 or more, such as from 7 to 14, and also more, such residues can be consecutive in such regions. The number, and also the length, of these contiguous regions depends on the total number of nucleotides and on the number of the novel residues in the oligonucleotide. Examples of possible arrangements are oligonucleotides having a total of 19 nucleotides, of which 5 at the 5' end and 5 in the 3'-half are modified in accordance with the invention, oligonucleotides having a total of 18 nucleotides of which 4 in the middle are modified in accordance with the invention, oligonucleotides having a total of 16 nucleotides, of which 10 in the middle are modified in accordance with the invention, and oligonucleotides having a total of 15 nucleotides, of which all are modified in accordance with the invention apart from one at the 3' end.

A preferred embodiment of the present invention is constituted by oligonucleotides in which at least two nucleoside residues of the formula Ia in which $R_1 = NH_2$ are present. Another preferred embodiment of the present invention is constituted by oligonucleotides in which at least two nucleoside residues of the formula Ia in which $R_1 = OH$ are present. A further preferred embodiment of the present invention is constituted by oligonucleotides in which at least two nucleoside residues of the formula Ib in which $R_1 = NH_2$ are present. Another preferred embodiment of the present invention is constituted by oligonucleotides in which at least two nucleoside residues of the formula Ib in which $R_1 = OH$ are present.

Due to their affinity for nucleic acids, the novel oligonucleotides have valuable biological activities and can be used as pharmaceutical active compounds or as diagnostic agents.

A further object of the present application is a process for preparing the novel oligonucleotides, which comprises introducing at least two derivatives of the formula IVa, IVb, IVc or IVd



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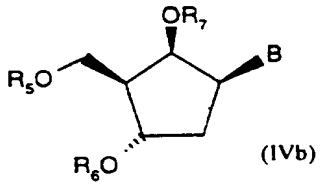

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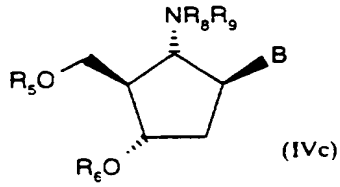
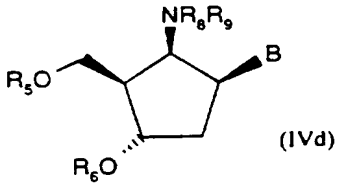


(IVc)



(IVd)

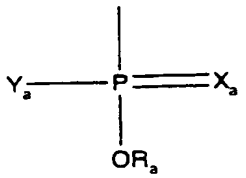
in which R_5 and R_7 are, independently of each other, a protecting group, R_8 and R_9 are, independently of each other, H or a protecting group, or R_8 and R_9 are bonded together in a single protecting group (e.g. a phthaloyl group), B has one of the meanings given above for the residues of the formulae Ia and Ib, and R_6 is a residue which forms a phosphorus-containing nucleotide-bridging group, into the synthesis.

Protecting groups, and methods for derivatizing the hydroxyl groups or the amino group with such protecting groups, are well known in sugar and nucleotide chemistry and also, for example, from peptide chemistry. Examples of such protecting groups are: benzyl, methylbenzyl, dimethylbenzyl, methoxybenzyl, dimethoxybenzyl, bromobenzyl, 2,4-dichlorobenzyl; diphenylmethyl, di(methylphenyl)methyl, di(dimethylphenyl)methyl, di(methoxyphenyl)methyl, di(dimethoxyphenyl)methyl, triphenylmethyl, tris-4,4',4''-tert-butylphenylmethyl, di-p-anisylphenylmethyl, tri(methylphenyl)methyl, tri(dimethylphenyl)methyl, methoxyphenyl(diphenyl)methyl, di(methoxyphenyl)phenylmethyl, tri(methoxyphenyl)methyl, tri(dimethoxyphenyl)methyl; triphenylsilyl, alkyldiphenylsilyl, dialkylphenylsilyl and trialkylsilyl having from 1 to 20, preferably from 1 to 12 and particularly preferably from 1 to 8 C atoms in the alkyl groups, for example trimethylsilyl, triethylsilyl, tri-n-propylsilyl, i-propyldimethylsilyl, t-butyldimethylsilyl, t-butyldiphenylsilyl, n-octyldimethylsilyl, (1,1,2,2-tetramethylethyl)-dimethylsilyl; -(C_1-C_8alkyl)$_2$Si-O-Si(C_1-C_8alkyl)$_2$-, in which alkyl is, for example, methyl, ethyl, n- and i-propyl, or n-, i- or t-butyl; C_2-C_{12}-, particularly C_2-C_8-acyl, for example acetyl, propanoyl, butanoyl, pentanoyl, hexanoyl, benzoyl, methylbenzoyl, methoxybenzoyl, chlorobenzoyl and bromobenzoyl; R_{S1}-SO_2-, in which R_{S1} is C_1-C_{12}alkyl, particularly C_1-C_6-alkyl, C_5- or C_6-cycloalkyl, phenyl, benzyl, C_1-C_{12}- and particularly C_1-C_4-alkylphenyl, or C_1-C_{12}- and particularly C_1-C_4-alkylbenzyl, or halophenyl or halobenzyl, for example methyl-, ethyl-, propyl-, butyl-, phenyl-, benzyl-, p-bromo-, p-methoxy- and p-methylphenylsulphonyl; C_1-C_{12}- which is unsubstituted or substituted by F, Cl, Br, C_1-C_4alkoxy, tri-(C_1-C_4alkyl)silyl or C_1-C_4alkylsulphonyl, preferably C_1-C_8-alkoxycarbonyl, for example methoxy-, ethoxy-, n- or i-propoxy- or n-, i- or t-butoxycarbonyl, 2-trimethylsilylethoxycarbonyl, 2-methylsulphonylethoxycarbonyl, allyloxycarbonyl or phenyloxycarbonyl or benzyloxycarbonyl which is unsubstituted or substituted as for alkoxycarbonyl, for example methyl- or methoxy- or chlorophenyloxycarbonyl or -benzyloxycarbonyl, and also 9-fluorenylmethyloxycarbonyl. If R_5 and/or R_7 is/are alkyl, it can be substituted by F, Cl, Br, C_1-C_4alkoxy, phenyloxy, chlorophenyloxy, methoxyphenyloxy, benzyloxy, methoxybenzyloxy or chlorophenyloxy. R_5 and R_7 in formulae IVa and IVb, or R_5, R_8 and R_9 in formulae IVc and IVd can be identical or different protecting groups.

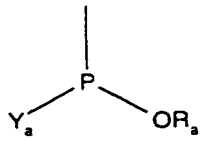
In a particularly preferred embodiment, R_5, R_7, R_8 and R_9 are, as protecting groups, benzyl, methylbenzyl, dimethylbenzyl, methoxybenzyl, dimethoxybenzyl, halogenated benzyl, in particular bromobenzyl; diphenylmethyl, di(methylphenyl)methyl, di(dimethylphenyl)methyl,

di(methoxyphenyl)methyl, di(methoxyphenyl)(phenyl)methyl, triphenylmethyl, tris-4,4',4''-tert-butylphenylmethyl, di-p-anisylphenylmethyl, tri(methylphenyl)methyl, tri(dimethylphenyl)-methyl, tri(methoxyphenyl)methyl, tri(dimethoxyphenyl)methyl; trimethylsilyl, triethylsilyl, tri-n-propylsilyl, i-propyl-dimethylsilyl, t-butyldimethylsilyl, t-butyldiphenylsilyl, n-octyldimethylsilyl, (1,1,2,2-tetramethylethyl)dimethylsilyl, $-(CH_3)_2Si-O-Si(CH_3)_2-$, $-(i-C_3H_7)_2Si-O-Si(i-C_3H_7)_2-$; acetyl, propanoyl, butanoyl, pentanoyl, hexanoyl, benzoyl, methylbenzoyl, methoxybenzoyl, chlorobenzoyl and bromobenzoyl; methyl-, ethyl-, propyl-, butyl-, phenyl-, benzyl-, p-bromo-, p-methoxy- and p-methylphenylsulphonyl; methoxy-, ethoxy-, n- or i-propoxy- or n-, i- or t-butoxycarbonyl, or phenyloxycarbonyl, benzyloxycarbonyl, methyl- or methoxy- or chlorophenyloxycarbonyl or -benzyloxycarbonyl or 9-fluorenylmethyloxycarbonyl.

As a phosphorus-containing radical which forms a nucleotide-bridging group, R_6 can correspond to the formula P1 or P2



(P1),



(P2)

in which Y_a is hydrogen, C_1-C_{12}alkyl, C_6-C_{12}aryl, C_7-C_{20}aralkyl, C_7-C_{20}alkaryl, $-OR_b$, $-SR_b$, $-NH_2$, primary amino, secondary amino, O^-M^+ or S^-M^+; X_a is oxygen or sulphur; R_a is hydrogen, M^+, C_1-C_{12}alkyl, C_2-C_{12}alkenyl or C_6-C_{12}aryl, or the group R_aO- is N-heteroaryl-N-yl having 5 ring members and from 1 to 3 N atoms; R_b is hydrogen, C_1-C_{12}alkyl or C_6-C_{12}-aryl; and M^+ is Na^+, K^+, Li^+, NH_4^+ or is primary, secondary, tertiary or quaternary ammonium; where alkyl, aryl, aralkyl and alkaryl in Y_a, R_a and R_b is unsubstituted or is substituted by alkoxy, alkylthio, halogen, -CN, $-NO_2$, phenyl, nitrophenyl or halophenyl.

As primary amino, Y_a preferably contains from 1 to 12 and particularly preferably from 1 to 6 C atoms, and as secondary amino, preferably from 2 to 12 and particularly preferably from 2 to 6 C atoms.

Primary amino and secondary amino can, for example, be radicals of the formula R_cR_dN, in which R_c is H or, independently, has the meaning of R_d, and R_d is C_1-C_{20}-, preferably C_1-C_{12}- and, particularly preferably, C_1-C_6-alkyl, C_1-C_{20}-, preferably C_1-C_{12}- and, particularly preferably, C_1-C_6-aminoalkyl, C_1-C_{20}-, preferably C_1-C_{12}- and, particularly preferably, C_1-C_6-hydroxyalkyl; carboxyalkyl or carbalkoxyalkyl, where the carbalkoxy group contains from 2 to 8 C atoms and the alkyl group contains from 1 to 6, preferably from 1 to 4 C atoms; C_2-C_{20}-, preferably C_2-C_{12}- and, particularly preferably, C_2-C_6-alkenyl; phenyl, mono- or di-(C_1-C_4-alkyl- or -alkoxy)phenyl, benzyl, mono- or di-(C_1-C_4-alkyl- or -alkoxy)benzyl; or 1,2-, 1,3- or 1,4-imidazolyl-C_1-C_6alkyl, or R_c and R_d are, together, tetra- or pentamethylene, 3-oxa-1,5-pentylene, $-CH_2-NR_e-CH_2CH_2-$ or $-CH_2CH_2-NR_{19}-CH_2CH_2-$, in which R_e is H or C_1-C_4alkyl. The amino group in the amino alkyl can be substituted by one or two C_1-C_4-alkyl or -hydroxyalkyl groups. The hydroxyl group in the hydroxyalkyl can be etherified with C_1-C_4-alkyl.

For Y_a in connection with the definition of M^+, primary, secondary, tertiary and quaternary ammonium is to be understood as meaning an ion of the formula $R_fR_gR_hR_iN^+$, in which R_f is C_1-C_{20}-, preferably C_1-C_{12}- and, particularly preferably, C_1-C_6-alkyl, -aminoalkyl, or -hydroxyalkyl; carboxyalkyl or carbalkoxyalkyl, where the carbalkoxy group contains from 2 to 8 C atoms and the alkyl group contains from 1 to 6, preferably from 1 to 4 C atoms; C_2-C_{20}-, preferably C_2-C_{12}- and, particularly preferably, C_2-C_6-alkenyl; phenyl, mono- or di-(C_1-C_4-alkyl- or -alkoxy)phenyl, benzyl, or mono- or di-(C_1-C_4-alkyl- or -alkoxy)benzyl; or 1,2-, 1,3- or 1,4-imidazolyl-C_1-C_6-alkyl, and R_g, R_h and R_i are, independently of each other, hydrogen or have the meaning of R_f, or R_f and R_g are, together, tetra- or penta-methylene, 3-oxa-1,5-pentylene, $-CH_2-NR_e-CH_2CH_2-$ or $-CH_2CH_2-NR_e-CH_2CH_2-$, in which R_e is H or C_1-C_4alkyl, and R_h and R_i, independently of each other, have the meaning of R_f. The amino group in the aminoalkyl can be substituted by one or two C_1-C_4-alkyl or -hydroxyalkyl groups. The hydroxyl group in the hydroxyalkyl can be etherified with C_1-C_4alkyl.

Examples of carboxyalkyl are carboxymethyl, carboxyethyl, carboxypropyl and carboxybutyl, and examples of carbalkoxyalkyl are these carboxyalkyl groups which are esterified with methyl or ethyl. Examples of alkenyl are allyl, but-1-en-3-yl or -4-yl, pent-3- or 4-en-1-yl or -2-yl, hex-3- or -4- or -5-en-1-yl or -2-yl. Examples of alkyl- and alkoxyphenyl or

-benzyl are methylphenyl, dimethylphenyl, ethylphenyl, diethylphenyl, methylbenzyl, dimethylbenzyl, ethylbenzyl, diethylbenzyl, methoxyphenyl, dimethoxyphenyl, ethoxyphenyl, diethoxyphenyl, methoxybenzyl, dimethoxybenzyl, ethoxybenzyl and diethoxybenzyl. Examples of imidazolylalkyl in which the alkyl group preferably contains from 2 to 4 C atoms are 1,2-, 1,3- or 1,4-imidazolylethyl or -n-propyl or -n-butyl. R_{19} is preferably H, methyl or ethyl.

Preferred examples of primary amino and secondary amino are methyl-, ethyl-, dimethyl-, diethyl-, di-i-propyl-, mono- or di-(1-hydroxy-eth-2-yl)-, phenyl- and benzylamino, acetylamino and benzoylamino, and also piperidinyl, piperazinyl and morpholinyl.

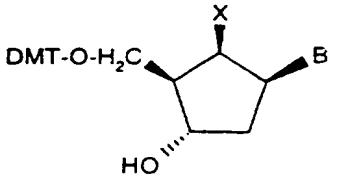
Preferred examples of primary and secondary ammonium are methyl-, ethyl-, dimethyl-, diethyl-, di-i-propyl-, mono- or di-(1-hydroxy-eth-2-yl)-, phenyl- and benzyl-ammonium.

Examples of Y_a, R_a and R_b as alkyl are methyl, ethyl and the isomers of propyl, butyl, pentyl, hexyl, heptyl and octyl; examples of Y_a, R_a and R_b as aryl are phenyl and naphthyl; examples of R_a as alkenyl are allyl and (C_1-C_4alkyl)CH=CH-CH_2-; examples of Y_a as aralkyl are phenyl-C_nH_{2n}- in which n is a number from 1 to 6, particularly benzyl; examples of Y_a as alkaryl are mono-, di- and tri(C_1-C_4alkyl)phenyl. Preferred substituents are chlorine, bromine, methoxy, -NO_2, -CN, 2,4-dichlorophenyl and 4-nitrophenyl. Examples of R_b are 2,2,2-trichloroethyl, 4-chlorophenyl, 2-chlorophenyl and 2,4-dichlorophenyl; and examples of R_bO- as N-heteroaryl are pyrrol-N-yl, triazol-N-yl and benzotriazol-N-yl.

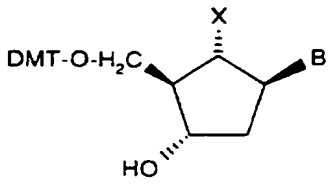
In a particularly preferred embodiment, R_a is β-cyanoethyl and Y_a is di(i-propylamino).

The novel oligonucleotides can be prepared by a variety of methods, in manners known per se, in commercially available DNA synthesizers which may or may not be automated and which are supplied together with method protocols. In the case of the bridging group -P(O)O^--, the phosphorotriester method, the phosphite triester method or the H-phosphonate method, with which the person skilled in the art is familiar, can be used, for example. In the phosphite triester method or the H-phosphonate method, the use of suitable sulphur-transferring reagents, with which the person skilled in the art is likewise familiar, leads, in the oxidation step, to the bridge grouping -P(O)S^--.

In the case of the phosphite triester method, the approach can be such, for example, that the nucleosides of the formulae IVa, IVb, IVc or IVd, in which R_5 and R_6 are each H, and R_7, R_8 and R_9 have the abovementioned meaning, are reacted with a protecting group reagent, for example 4,4'-dimethoxytriphenylmethyl chloride, to form a nucleoside of the formula A or C,

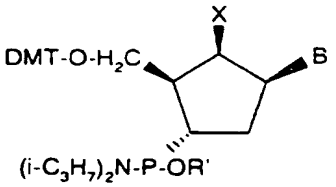


(A)

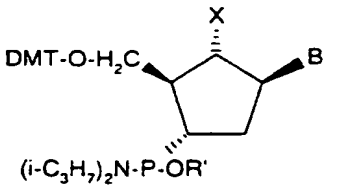


(C)

where X is OR_7 or NR_8R_9, and R_7, R_8 and R_9 have the abovementioned meaning. The compounds of the formulae A and C can be bound by a linker, for example succinic anhydride, to a solid support material, for example to controlled pore glass (CPG), which contains long-chain alkylamino groups. In a separate procedure, the hydroxyl group of the compounds of the formulae A and C is derivatized, for example into a phosphoramidite using $R'OP[N(i\text{-propyl})_2]_2$, in order to form compounds of the formulae D and E,



(D)



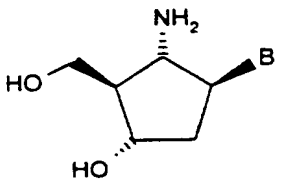
(E)

where X has the abovementioned meaning and R' is, for example, β-cyanoethyl.

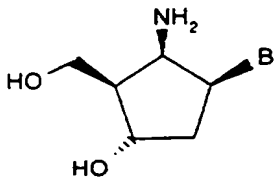
After the protecting group, for example the DMT group, of the material bound to the support has been removed, a compound of the formula D or E is coupled on, with elimination of $-N(i\text{-}C_3H_7)_2$, any free hydroxyl groups which are present are blocked (capping) and the phosphite which has been formed is then oxidized to the phosphate. After the dimer has been deprotected, the reaction cycle is repeated with a compound of the formulae D or E

until an oligomer has been synthesized which has the desired number of monomer units, and the product is then released from the support material. In this way, oligonucleotides are obtained in which all the nucleoside residues consist of residues of the formulae Ia or Ib. It is also possible to prepare oligonucleotides having any monomer units in any sequence in this way, depending on the use of synthetic, natural and novel nucleoside building blocks in the individual reaction cycles.

The invention furthermore relates to nucleosides of the formula IVc' or IVd'



(IVc')

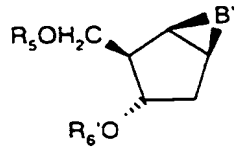


(IVd')

in which B has one of the meanings given above for the residues of the formulae Ia and Ib. They have antiviral and anti-proliferative properties and can accordingly be used as pharmaceuticals, for example as therapeutic agents.

The present invention also relates to a process for preparing compounds of the formula IVc' or IVd', which comprises reacting

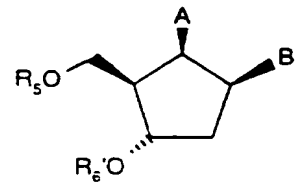
(a) a compound of the formula V



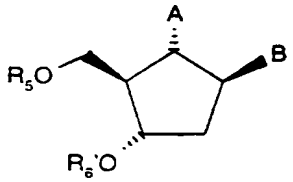
(V)

in which R_5 and R_6' are, independently of each other, a protecting group, and B' is a pyrimidine residue which is bonded via N and O, or

(b) a compound of the formula (VIa) or (VIb),

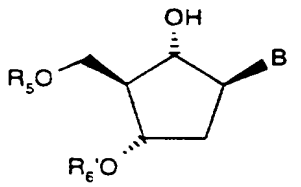


(VIa)

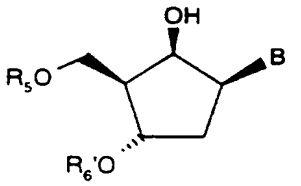


(VIb)

in which R_5 and R_6' are, independently of each other, a protecting group, B has one of the abovementioned meanings, and A is a customary leaving group, for example a tosylate or a mesylate, which is obtainable by converting, in a known manner, the free OH group of a compound of the formula IVa' or IVb'



(IVa')



(IVb')

in which R_5 , R_6' and B are defined as above, into a leaving group A, for example a tosylate or a mesylate,

with a metal azide, for example NaN_3, reducing the azido group to the amino group and removing the protecting groups. The reactions are customarily effected in the presence of a solvent, at standard pressure and at a temperature of from 20 to 200°C. The reduction is advantageously effected catalytically. The Lindlar catalyst is an example of a suitable catalyst. The O-5' and O-3'-protected derivatives which are obtained in this way can be converted, in a well known manner, into the compounds IVc' and IVd' by eliminating the protecting groups R_5 and R'_6. Thus, the compound IVc' can, for example, be readily prepared from the compound A21 by removing the benzyl protecting groups by means of catalytic hydrogenation over 10% Pd-C. Alternatively, IVc' can also be prepared from A23 be removing the trifluoroacetyl protecting group under basic conditions.

The novel oligonucleotides have an increased stability towards degradation by nucleases and their pairing with complementary RNA is improved. What is particularly surprising, however, is that the novel oligonucleotides possess a significantly higher antisense activity in cellular experiments than that of oligonucleotides which contain natural nucleosides in place of the residues Ia or Ib. The novel oligonucleotides are consequently particularly suitable for antisense technology, that is for inhibiting the expression of undesirable protein products by means of binding to suitable, complementary nucleotide sequences of mRNA (EP266,099, WO87/07300 and WO89/08146). They can be employed for treating infections and diseases, for example by blocking the expression of bioactive proteins at the level of the nucleic acids (for example oncogenes). The novel oligonucleotides are also suitable for use as diagnostic agents and can be used as gene probes for detecting viral infections or genetically determined diseases by means of selective interaction at the level of single-stranded or double-stranded nucleic acids (gene probes).

The invention furthermore relates to the use of the novel oligonucleotides as diagnostic agents for detecting viral infections or genetically determined diseases.

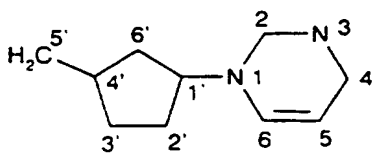
The invention also relates to the novel nucleosides of the formulae IVc' and IVd', and also the novel oligonucleotides, for use in a therapeutic process for treating diseases in warm-blooded animals including man. When they are being administered to warm-blooded animals of about 70 kg bodyweight, the dose can, for example, be from 0.01 to 1000 mg

per day. The administration is preferably effected parenterally, for example intravenously or intraperitoneally, in the form of pharmaceutical preparations.

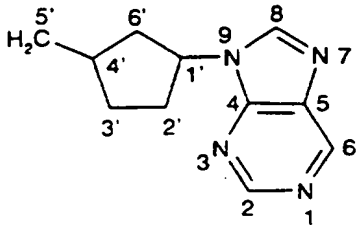
The invention furthermore relates to a pharmaceutical preparation which comprises an effective quantity of a nucleoside of the formula IVc' or IVd', or of a novel oligonucleotide, alone or together with other active compounds, a pharmaceutical excipient material, preferably in a significant quantity, and, if desired, auxiliary substances.

The pharmacologically active inventive nucleosides of the formula IVc or IVd, and the inventive oligonucleotides, can be used in the form of preparations which can be administered parenterally, or in the form of infusion solutions. Preferably, such solutions are isotonic, aqueous solutions or suspensions, with it being possible, for example in the case of lyophilized preparations which comprise the active substance alone or together with an excipient material, for example mannitol, for these solutions or suspensions to be prepared prior to use. The pharmaceutical preparations can be sterilized and/or comprise auxiliary substances, for example preservatives, stabilizers, wetting agents, emulsifiers, solubilizers, salts for regulating the osmotic pressure and/or buffers. The pharmaceutical preparations, which can, if desired, comprise additional pharmacologically active compounds, for example antibiotics, are prepared in a manner known per se, for example using conventional dissolution or lyophilization methods, and comprise from about 0.1 % to 90 %, in particular from about 0.5 % to about 30 %, for example from 1 % to 5 %, of active compound(s).

The following examples illustrate the invention. The ^{1}H-NMR spectra are based on the following numbering of the atoms:



and

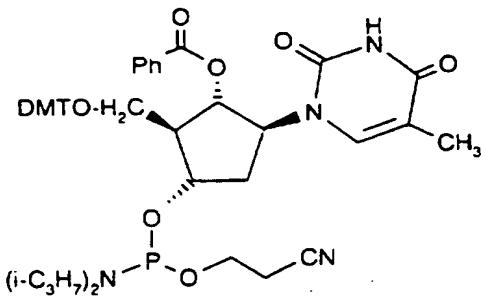


Abbreviations used in the text and in the formulae:

Bn	benzyl
Bz	benzoyl
DMF	N,N-dimethylformamide
DMT	4,4'-dimethoxytrityl
Ph	phenyl
RT	room temperature
TBDMS	tert-butyldimethylsilyl
THF	tetrahydrofuran

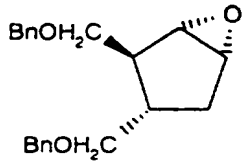
A) Preparation of nucleoside analogues

Example A1: Preparation of compound No. A6



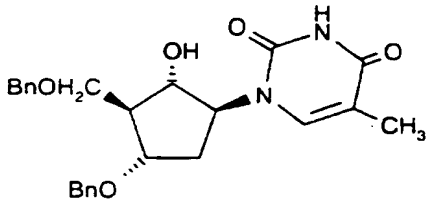
(A6)

(a1) 5.5 ml of boron trifluoride etherate are added dropwise, at 0°C and within the space of 10 min, to a solution of 6.8 g of compound No. A1



(A1)

prepared in accordance with Biggadike, K., et al., J. Chem. Soc., Perkin Trans. I, 549-554 (1988) and 23.65 g of bis(trimethylsilyl)thymine in 200 ml of dichloroethane. The solution is subsequently stirred at 32°C for 6 h and then cooled to 5°C; 10 g of ice are then added to it. After the solution has been stirred for 10 minutes, 200 ml of a saturated aqueous solution of $NaHCO_3$ are added and the whole is filtered through Hyflo. The filtrate is extracted 3 times with 200 ml of methylene chloride on each occasion and the combined organic extracts are dried over magnesium sulphate and concentrated by evaporation. Chromatography of the residue through silica gel using ethyl acetate as the eluent yields 5.58 g of compound (A2).

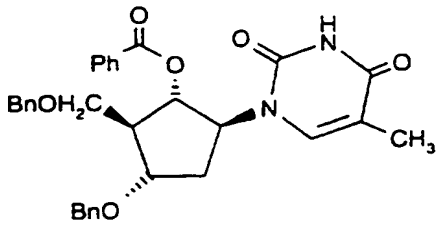


(A2)

^{1}H-NMR (250 MHz, $CDCl_3$, TMS): δ = 10.0 [s, 1H, NH]; 7.0 [s, 1H, H-C(6)]; 4.75 [q, 1H, H-C(1')]; 4.50 [m, 4H, CH_2, Bn]; 3.90 [m, 1H, H-C(3']; 1.80 [s, 3H, CH_3-C(5)].

(b1) 1.61 ml of pyridine, 1.41 g of benzoic anhydride and a spatula tip of 4-(N,N-dimethyl-amino)pyridine (DMAP) are added to a solution of 2.1 g of the compound A2 in 50 ml of methylene chloride. The solution is stirred at RT. After 16 h, a further 0.141 g of benzoic anhydride and a further spatula tip of DMAP are added, and the reaction mixture is heated at 35°C for 3 h. At the end of this period, a further 0.2 g of DMAP are added and the mixture is stirred at RT for a further 2 h. 5 ml of methanol are then added, and the solution is stirred for 15 min and then poured into a mixture consisting of 100 ml of methylene

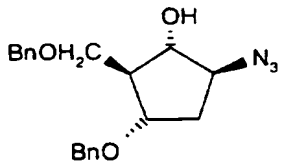
chloride and 50 ml of a saturated, aqueous solution of $NaHCO_3$. The organic phase is separated off and the aqueous phase is extracted once again with 50 ml of methylene chloride. The combined organic extracts are dried over magnesium sulphate and concentrated by evaporation. Chromatography of the residue on silica gel using diethyl ether as the eluent yields 2.30 g of the compound A3.



(A3)

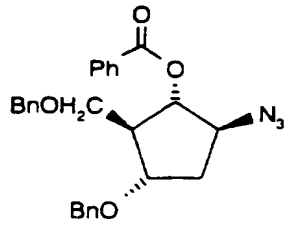
^{1}H-NMR (250 MHz, $CDCl_3$, TMS): δ = 8.95 [s, 1H, NH]; 8.0 [d, 2H, H-C(ar), Bz]; 7.10 [s, 1H, H-C(6)]; 5.55 [m, 1H]; 5.40 [m, 1H]; 4.50 [m, 4H, CH_2, Bn]; 4.10 [m, 1H, H-C(3')]; 1.70 [s, 3H, CH_3-C(5)].

(a2) 1.0 ml of triethylamine and 0.767 ml of benzoyl chloride are added, at 0°C, to a solution of 2.12 g of compound A7



(A7)

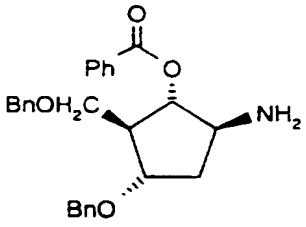
prepared from compound A1 in accordance with Biggadike, K., et al., J. Chem. Soc., Perkin Trans. I, 549-554 (1988) in 20 ml of methylene chloride. The solution is stirred at RT for 18 h and then diluted with a mixture consisting of 20 ml of methylene chloride and 50 ml of water. The organic phase is separated off and the aqueous phase is extracted a further 2 times with 20 ml of methylene chloride on each occasion. The combined organic extracts are dried over magnesium sulphate and concentrated by evaporation. Chromatography on silica gel using methylene chloride as the eluent, and re-chromatograpy of the mixed fractions using methylene chloride/hexane, 3/1, yields 2.33 g of the compound A8.



(A8)

^{1}H-NMR (250 MHz, $CDCl_3$, TMS): δ = 8.05 [d, 2H, H-C(ar), Bz]; 5.25 [t, 1H, H-C(6')]; 4.50 [m, 4H, CH_2, Bn]; 4.25 [m, 1H, H-(C-3')]; 3.95 [m, 1H, H-(C-1')]; 3.70 [m, 1H, H-(C-5')]; 3.60 [m, 1H, H-(C-5')]. (Numbering as for nucleoside analogues, see above).
MS: 428 [$(M-N_2)^+$].

(b2) 2.31 g of the compound A8 are hydrogenated over Lindlar catalyst, at atmospheric pressure and at RT, for 6 h in 20 ml of toluene/methanol, 1/1. After the catalyst has been filtered off, the solution is concentrated by evaporation and the residue is purified by chromatography on silica gel using ethyl acetate/methanol, 4/1, as the eluent. 2.15 g of the compound A9 are obtained.

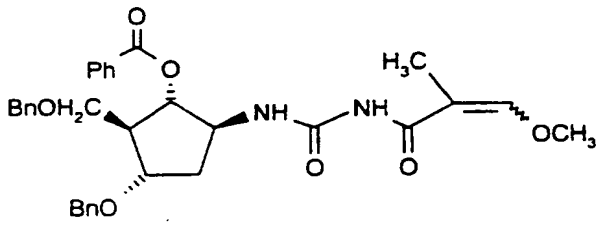


(A9)

^{1}H-NMR (250 MHz, $CDCl_3$, TMS): δ = 8.05 [d, 2H. H-C(ar), Bz]; 4.95 [t, 1H, H-C(6')]; 4.50 [m, 4H, CH_2, Bn]; 4.00 [m, 1H, H-(C-3')]; 3.65 [m, 3H, H_2-(C-5') and H-(C-1')]; 1.95 [s, broad, 2H, NH_2]. (Numbering as for nucleoside analogues, see above).

(c2) 0.813 ml of ß-methoxymethacryloylisocyanate is added, at -60°C, to a solution of 2.14 g of compound A9 in 20 ml of methylene chloride. The cold bath is then removed and the reaction mixture is allowed to warm to RT. After 1.5 h, the reaction mixture is poured into a mixture consisting of 50 ml of methylene chloride and 50 ml of a saturated, aqueous

solution of $NaHCO_3$; the organic phase is separated off and the aqueous phase is extracted a further 2 times with 20 ml of methylene chloride on each occasion. The combined organic extracts are dried over magnesium sulphate and concentrated by evaporation. The residue is purified by chromatography on silica gel using methylene chloride/dimethyl ether, 1/1, as the eluent. 2.75 g of the compound A10 are obtained.

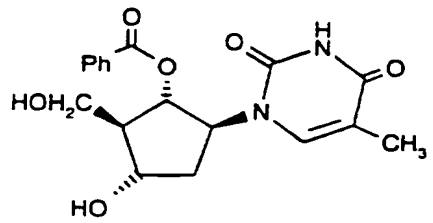


(A10)

^{1}H-NMR (250 MHz, $CDCl_3$, TMS): δ = 9.15 [d, 1H, NH]; 8.95 s, [broad, 1H, NH]; 8.00 [d, 2H, H-C(ar), Bz]; 5.25 [t, 1H, H-C(6')]; 4.70 [m, 1H, H-C(1')]; 4.50 [m, 4H, CH_2, Bn]; 4.00 [m, 1H, H-(C-3')]; 3.75 [s, 3H, OCH_3]; 3.65 [m, 2H, H_2-(C-5')]; 1.75 [s, 3H, CH_3]. (Numbering as for nucleoside analogues, see above).

(d2) A solution of compound A10 in a mixture composed of 43 ml of ethanol and 4.8 ml of 2N aqueous HCl is heated to reflux for 24 h. The ethanol is then removed on a rotary evaporator and the solution which remains is treated with a mixture composed of 50 ml of diethyl ether and 50 ml of water. The organic phase is separated off and the aqueous phase is extracted a further 2 times with 50 ml of diethyl ether on each occasion. The combined organic extracts are dried over magnesium sulphate and concentrated by evaporation. Chromatography of the residue on silica gel using methylene chloride/diethyl ether, 1/1, as the eluent yields 1.92 g of compound A3.

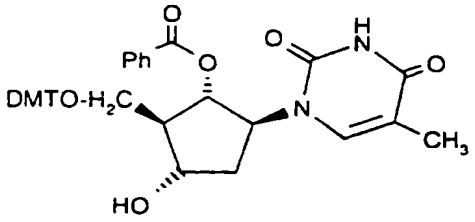
(c) 2,50 g of compound A3 are hydrogenated over 10 % Pd/C, at RT and at atmospheric pressure, for 2.5 h in ethyl acetate/methanol, 1/1. After the catalyst has been filtered off, the solution is concentrated by evaporation and the residue is recrystallized from ethanol. 1.30 g of the compound A4 are obtained.



(A4)

^{1}H-NMR (250 MHz, D_2O, TMS): δ = 7.90 [d, 2H, H-C(ar)]; 5.45 [t, 1H, H-C(6')]; 5.05 [q, 1H, H-C(1')]; 1.80 [s, 3H, CH_3-C(5)].

(d) 0.447 ml of triethylamine, 0.940 g of DMT chloride and a spatula tip of 4-(N,N-dimethylamino)pyridine are added, at 0°C, to a solution of 0.830 g of compound A4 in 23 ml of pyridine. The solution is then stirred at ice-bath temperature for 18 h and subsequently diluted with 200 ml of ethyl acetate. After having been extracted three times with 50 ml of water on each occasion, the organic phase is dried over magnesium sulphate and then concentrated by evaporation. Chromatography of the residue through silica gel using diethyl ether/triethylamine, 99/1, as the eluent yields 0.913 g of compound A5.



(A5)

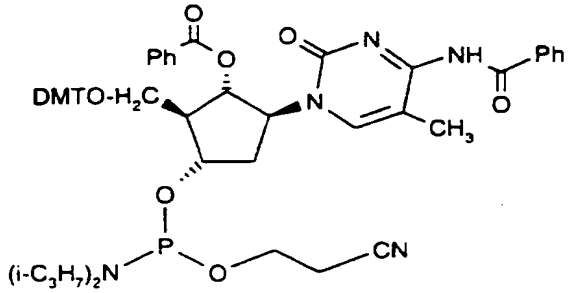
^{1}H-NMR (250 MHz, $CDCl_3$, TMS): δ = 8.85 [s, 1H, NH]; 8.00 [d, 2H, H-C(ar), Bz]; 7.00 [s, 1H, H-C(6)]; 6.80 [d, 4H, H-C(ar), DMT]; 5.50 [t, 1H, H-C(6')]; 5.10 [q, 1H, H-C(1')]; 4.40 [m, 1H, H-C(3')]; 3.75 [s, 6H, OCH_3]; 1.80 [s, 3H, CH_3-C(5)].

(e) 0.913 g of compound A5 is added to an initially introduced mixture composed of 0.237 g of diisopropylammonium tetrazolide, 0.457 g of 2-cyanoethyl-N,N,N',N'-tetraisopropylphosphorodiamidite and 30 ml of methylene chloride. The reaction mixture is stirred at room temperature for 4 hours and, after that, is poured into a mixture composed of 100 ml of methylene chloride and 50 ml of a saturated, aqueous solution of $NaHCO_3$. The organic

phase is separated off and the aqu ous solution is then extracted a further 2 times with 50 ml of methylene chloride on each occasion. The combined organic extracts are dried with $MgSO_4$ and concentrated by evaporation. The residue is chromatographed through silica gel (toluene/ethyl acetate, 1:1, containing 2 % added triethylamine). The resulting foam is dissolved in 2 ml of methylene chloride and this solution is added dropwise, at 0°C, to 100 ml of pentane. 0.849 g of compound A6 is obtained (diastereoisomers, 1:1).

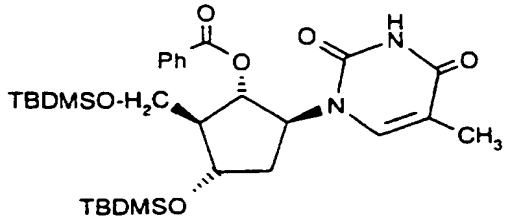
^{1}H-NMR (250 MHz, $CDCl_3$, TMS): δ = 7.95 [d, 2H, H-C(ar), Bz)]; 6.74 [m, 4H, H-C(ar), DMT]; 5.60 [m, 1H, H-C(1')]; 5.25 [m, 1H, H-(C-3')]; 1.88 [s, 3H, CH_3-(C-5)].
^{31}P-NMR($CDCl_3$): 148.202 and 147.903.

Example A2: Preparation of compound No. A18



(A18)

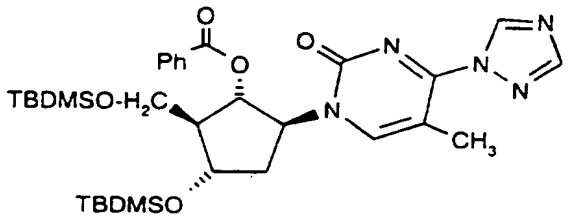
(a) 1.17 g of TBDMS chloride and 1.17 g of imidazole are added to a solution of compound A4 in 20 ml of DMF. The solution is stirred at RT for 18 h and is then diluted with 100 ml of diethyl ether and subsequently extracted 3 times with 50 ml of ice water on each occasion. The organic phase is dried over magnesium sulphate and concentrated by evaporation. The residue is purified by chromatography on silica gel using methylene chloride/diethyl ether/ triethylamine, 4/1/0.05, as the eluent. 1.69 g of the compound A12 are obtained.



(A12)

^{1}H-NMR (250 MHz, $CDCl_3$,TMS): δ = 9.25 [s, 1H, NH]; 7.95 [d, 2H, H-C(ar), Bz]; 7.0 [s, 1H, H-C(6)]; 5.40 [t, 1H]; 5.30 [m, 1H]; 4.30 [m, 1H, H-C(3')]; 1.85 [s, 3H, CH_3-C(5)]; 0.85 [s, 18H, $(CH_3)_3C$].
MS: 531 ([M-tert-butyl]$^+$)

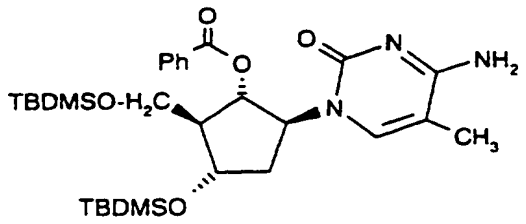
(b) 0.658 ml of $POCl_3$ is added dropwise, at 0°C and within the space of 5 min, to a solution of 1.69 g of compound A12, 4.47 g of 1,2,4-triazole and 9.15 ml of triethylamine in 25 ml of acetonitrile. The mixture is stirred at RT for 2.5 h and then poured into 30 ml of methylene chloride/triethylamine, 1/1, and the whole is subsequently extracted once with 50 ml of a saturated, aqueous solution of $NaHCO_3$ and then 2 times with 50 ml of water on each occasion. The organic phase is dried over magnesium sulphate and concentrated by evaporation. The residue is purified by chromatography on silica gel using methylene chloride/diethyl ether/triethylamine, 2/1/0.03, as the eluent. 1.73 g of the compound A13 are obtained.



(A13)

^{1}H-NMR (250 MHz, $CDCl_3$,TMS): δ = 9.15 [s, 1H, triazole]; 8.00 [s, 1H, triazole]; 7.90 [d, 2H, H-C(ar), Bz]; 7.60 [s, 1H, H-C(6)]; 5.55 [t, 1H]; 5.25 [m, 1H]; 4.35 [m, 1H, H-C(3')]; 2.35 [s, 3H, CH_3-C(5)]; 0.80 [s, 18H, $(CH_3)_3C$].

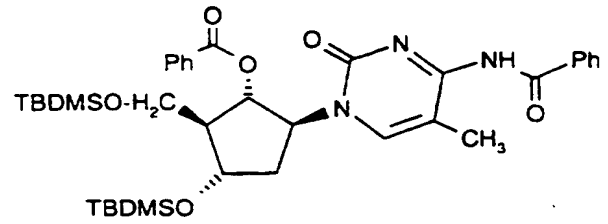
(c) The product obtained in Example A2(b) is dissolved in 21 ml of dioxane and the mixture is stirred at RT for 4 h after having added 7 ml of conc. ammonia. The dioxane is then removed on a rotary evaporator and the aqueous solution which remains is stirred up with 100 ml of ethyl acetate. After the aqueous phase has been removed, the ethyl acetate phase is extracted a further 3 times with 50 ml of water on each occasion, dried over magnesium sulphate and then concentrated by evaporation. The residue is purified by chromatography on silica gel using ethyl acetate/methanol/triethylamine, 9/1/0.09, as the eluent. 1.45 g of the compound A14 are obtained.



(A14)

^{1}H-NMR (250 MHz, $CDCl_3$, TMS): δ = 7.95 [d, 2H, H-C(ar), Bz]; 7.05 [s, 1H, H-C(6)]; 5.50 [t, 1H]; 5.30 [m, 1H]; 4.25 [m, 1H, H-C(3')]; 1.80 [s, 3H, CH_3-C(5)]; 0.85 [s, 18H, $(CH_3)_3C$].
MS: 588 ([M+H]$^+$)

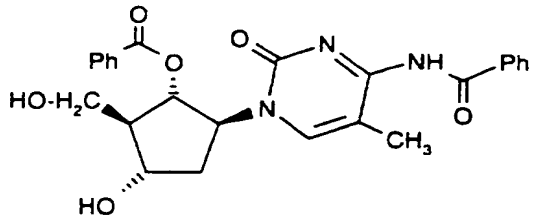
(d) 0.340 ml of benzoyl chloride, 0.506 ml of triethylamine and 1 spatula tip of 4-(N,N-dimethylamino)pyridine are added, at 0°C, to a solution of 1.43 g of compound A14 in 30 ml of diethyl ether. After having been stirred at RT for 1 h, the reaction mixture is poured into a mixture composed of 100 ml of diethyl ether and 50 ml of a saturated, aqueous solution of $NaHCO_3$, and the aqueous phase is separated off and the organic phase is washed a further 2 times with water. The organic phase is dried over magnesium sulphate and concentrated by evaporation. The residue is purified by chromatography on silica gel using methylene chloride/triethylamine, 99/1, as the eluent. 1.56 g of the compound A15 are obtained.



(A15)

^{1}H-NMR (250 MHz, $CDCl_3$, TMS): δ = 13.15 [s, broad, 1H, NH-Bz]; 8.20 [d, 2H]; 7.90 [d, 2H]; 7.15 [s, 1H, H-C(6)]; 5.40 [t, 1H]; 5.25 [m, 1H]; 4.25 [m, 1H, H-C(3')]; 2.0 [s, 3H, CH_3-C(5)]; 0.85 [s, 18H, $(CH_3)_3C$].
MS: 691 (M$^+$)

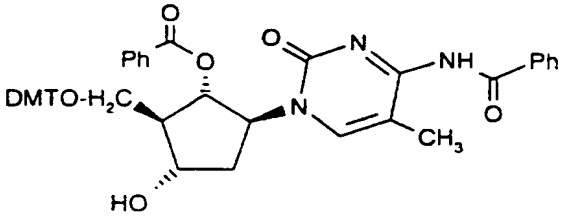
(e) 6.96 ml of a 1 M solution of tetrabutylammonium fluoride in THF are added to a solution of 1.204 g of compound A15 in 10 ml of THF. After the mixture has been stirred at RT for 2 h, it is evaporated to dryness and the residue is purified by chromatography on silica gel using ethyl acetate/methanol, 9/1, as the eluent. 0.879 g of the compound A16 is obtained.



(A16)

^{1}H-NMR (250 MHz, CD_3OD, TMS): δ = 8.20 [d, 2H]; 7.95 [d, 2H]; 7.70 [s, 1H, H-C(6)]; 5.55 [t, 1H]; 5.20 [m, 1H]; 4.30 [m, 1H, H-C(3')]; 3.70 [m, 2H, C(5')-H_2]; 2.05 [s, 3H, CH_3-C(5)].

(f) The compound A17 is prepared in analogy with the preparation of compound A5.



(A17)

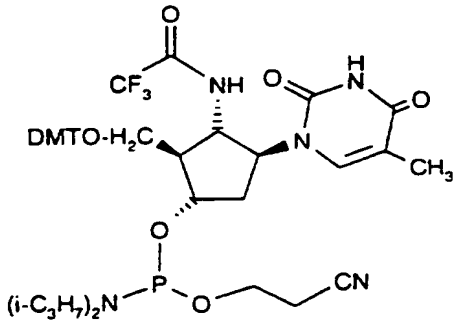
^{1}H-NMR (250 MHz, $CDCl_3$, TMS): δ = 13.,5 [s, broad, 1H, NH-Bz]; 8.30 [d, 2H]; 7.95 [d, 2H]; 6.80 [d, 4H, H-C(ar), DMT]; 5.55 [t, 1H]; 5.15 [m, 1H]; 4.45 [m, 1H, H-C(3')]; 3.70 [s, 6H, OCH_3]; 2.0 [s, 3H, CH_3-C(5)].

(g) The compound A18 is prepared in analogy with the preparation of compound A6 (2 diastereoisomers).

^{1}H-NMR (250 MHz, $CDCl_3$, TMS): δ = 13.2 [s, broad, 1H, NH-Bz]; 8.25 [d, 2H]; 7.95 [d, 2H]; 6.65 [m, 4H, H-C(ar), DMT]; 5.55 [m, 1H]; 5.20 [m, 1H]; 4.55 [m, 1H, H-C(3')]; 1.95 [s, 3H, CH_3-C(5)].

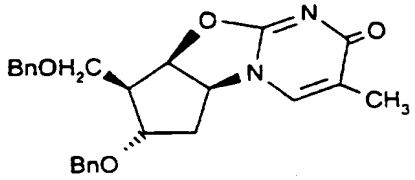
^{31}P-NMR ($CDCl_3$): 148.371 and 147.934.

Example A3: Preparation of compound No. A25



(A25)

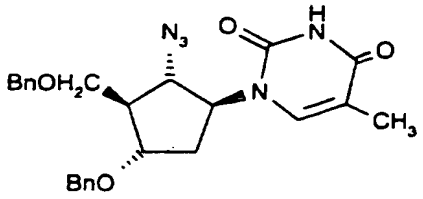
(a) 1.77 g of compound A2 and 1.74 g of diphenyl carbonate are heated at 150°C for 30 min in 50 ml of DMF and in the presence of a spatula tip of $NaHCO_3$. The solvent is then evaporated off and the residue is purified by chromatography on silica gel using ethyl acetate/ methanol, 4/1, as the eluent.The product-containing fractions are subsequently recrystallized from ethyl acetate. 1.29 g of the compound A19 are obtained.



(A19)

^{1}H-NMR (250 MHz, CD_3OD, TMS): δ = 7.40 [s, 1H, H-C(6)]; 5.35 [t, 1H, H-C(6')]; 4.85 [m, 1H, H-C(1')]; 4.35 [m, 4H, CH_2-benzyl]; 3.70 [m, 1H, H-C(3']; 1.8 [s, 3H, CH_3-C(5)].

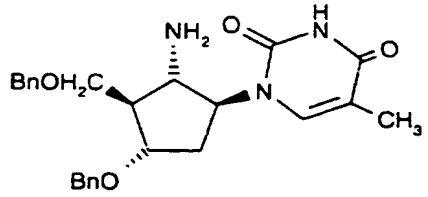
(b) 3.68 g of compound A19 and 0.859 g of NaN_3 are heated at 125°C for 21 h in 40 ml of DMF. The reaction mixture is then poured into 300 ml of diethyl ether and the ethereal solution is extracted 3 times with 100 ml of water on each occasion. The organic phase is subsequently dried over magnesium sulphate and concentrated by evaporation. The residue is purified by chromatography on silica gel using diethyl ether as the eluent. 1.78 g of the compound A20 are obtained.



(A20)

^{1}H-NMR (250 MHz, $CDCl_3$, TMS): δ = 9.95 [s, 1H, NH]; 6.95 [s, 1H, H-C(6)]; 4.55 [m, 5H, CH_2, Bn, and H-C(1')]; 4.15 [t, 1H, H-C(6')]; 4.00 [m, 1H, H-C(3')]; 1.90 [s, 3H, CH_3-C(5)].
MS: 479 ([M + NH_4]$^+$).
IR: 2110 cm^{-1}, 1690 cm^{-1}.

(c) 0.833 g of compound A20 is hydrogenated over 0.177 g of Lindlar catalyst, at atmospheric pressure and at RT, for 10 h in 20 ml of methanol/ethyl acetate, 1/1, with 0.9 ml of 2 N aqueous HCl being added after 8 h. After the catalyst has been filtered off, the solution is concentrated by evaporation; the residue is dissolved in 20 ml of methanol and saturated, aqueous $NaHCO_3$ solution is added. The mixture is then concentrated by evaporation once more and the residue is treated a further 3 times with fresh methanol; the mixture is concentrated by evaporation again. Finally, the residue is purified by chromatography on silica gel using ethyl acetate/methanol, 2/1, as the eluent. 0.777 g of the compound A21 are obtained.

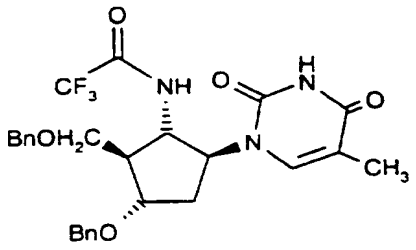


(A21)

^{1}H-NMR (250 MHz, $CDCl_3$, TMS): δ = 6.95 [s, 1H, H-C(6)]; 4.45 [m, 4H, CH_2, Bn]; 3.90 [m, 1H, H-C(3')]; 3.65 [m, 2H, C(5')-H_2]; 3.30 [t, 1H, H-C(6')]; 1.90 [s, 3H, CH_3-C(5)].
MS: 436 ([M + H]$^+$).

(d) 0.592 ml of trifluoroacetic anhydride is added dropwise, at -78°C and within the space of 3 min, to a solution of 1.48 g of compound A21 and 0.688 ml of pyridine in 30 ml of

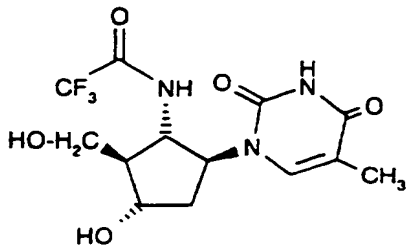
methylene chloride. The reaction mixture is allow d to warm to RT within the space of 1 h and is then poured into 200 ml of diethyl ether; the ethereal solution is then extracted 3 times with 50 ml of water on each occasion. The organic phase is then dried over magnesium sulphate and concentrated by evaporation. The residue is dissolved in 30 ml of dioxane, after which 10 ml of conc. ammonia are added and the solution is stirred at RT for 10 min. Subsequently, the mixture is concentrated down to a volume of approximately 10 ml by evaporating off the dioxane, after which 100 ml of diethyl ether are added and the whole is extracted 3 times with 30 ml of water on each occasion. After the organic phase has been dried over magnesium sulphate and the solvent has been removed, the residue is purified by chromatography on silica gel using diethyl ether as the eluent. 0.849 g of the compound A22 is obtained.



(A22)

^{1}H-NMR (250 MHz, $CDCl_3$, TMS): δ = 9.85 [s, 1H, NH]; 7.75 [d, 1H, NH]; 7.05 [s, 1H, H-C(6)]; 5.35 [m, 1H, H-C(1')]; 4.50 [m, 5H, CH_2, Bn, and H-C(6')]; 4.05 [m, 1H, H-C(3')]; 1.80 [s, 3H, CH_3-C(5)].

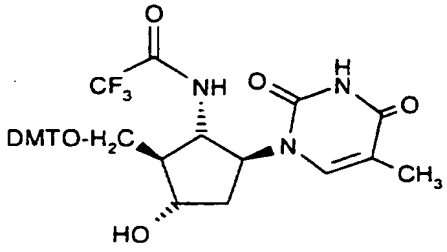
(e) 1.84 g of compound A22 are hydrogenated over 0.346 g of 10 % Pd/C, at atmospheric pressure and at RT, for 3 h in 40 ml of ethyl acetate/methanol, 1/1. 1.28 g of the compound A23 are obtained after filtering off the catalyst and concentrating the filtrate by evaporation.



(A23)

^{1}H-NMR (250 MHz, CD_3OD, TMS): δ = 7.40 [s, 1H, H-C(6)]; 4.90 [q, 1H, H-C(1')]; 4.35 [m, 1H]; 4.15 [m, 1H]; 1.80 [s, 3H, CH_3-C(5)].
MS: 351 ([M + H]$^+$).

(f) Compound A24 is prepared in analogy with compound (A5).



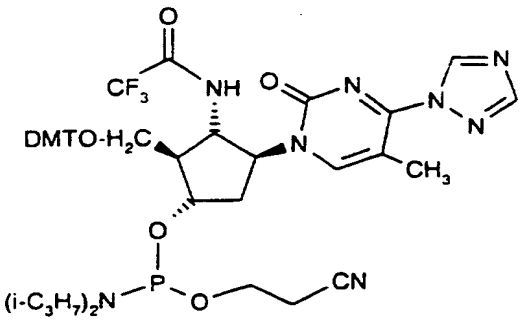
(A24)

^{1}H-NMR (250 MHz, $CDCl_3$, TMS): δ = 7.05 [s, 1H, H-C(6)]; 5.35 [m, 1H, H-C(1')]; 4.30 [m, 2H, H-C(6') and H-C(3')]; 3.80 [s, 6H, OCH_3]; 3.35 [m, 2H, C(5')-H_2]; 1.80 [s, 3H, CH_3-C(5)].

(g) Compound A25 is prepared in analogy with compound A6 (2 diastereoisomers).

^{1}H-NMR (250 MHz, $CDCl_3$, TMS): δ = 9.35 [s, broad, 1H, NH]; 7.65 ["t", 1H, NH]; 7.10 ["d", 1H, H-C(6)]; 6.70 ["d", 4H, H-C(ar), DMT]; 5.30 [m, 1H, H-C(1')]; 4.60 - 4.30 [m, 2H, H-C(6') and H-C(3')]; 3.80 [s, 6H, OCH_3]; 3.35 [m, 2H, 2 x H-$C(CH_3)_2$]; 1.90 [s, 3H, CH_3-C(5)].
^{31}P-NMR ($CDCl_3$): 147.873 and 147.604.

Example A4: Preparation of compound No. A26

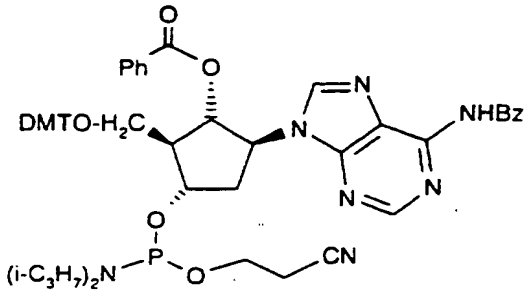


(A26)

0.197 ml of $POCl_3$ is added dropwise, at 10°, to a solution of 2.75 ml of triethylamine in 20 ml of acetonitrile/methylene chloride, 1/1. The mixture is stirred at RT for 30 min and a solution of compound A25 in 2 ml of methylene chloride is then added dropwise. After it has been stirred at RT for 3 h, the reaction mixture is poured into a mixture composed of 100 ml of methylene chloride and 5 ml of triethylamine, and the whole is extracted 3 times with 50 ml of ice water on each occasion. After the organic phase has been dried over magnesium sulphate and the solvent has been evaporated off, the residue is purified by chromatography on silica gel using ethyl acetate/ether/triethylamine, 9/1/0.01, as the eluent. 0.647 g of compound A26 are obtained.

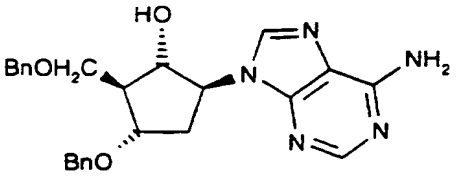
^{1}H-NMR (250 MHz, $CDCl_3$, TMS): δ = 9.25 ["d", 1H, CH, triazole]; 8.10 ["d", 1H, CH, triazole]; 7.70 ["d", 1H, H-C(6)]; 6.70 ["d", 4H, H-C(ar), DMT]; 5.30 [m, 1H, H-C(1')]; 4.70 - 4.30 [m, 2H, H-C(6') and H-C(3')]; 3.75 [s, 6H, OCH_3]; 2.40 ["d", 3H, CH_3-C(5)].
^{31}P-NMR ($CDCl_3$): 147.764 and 147.455.

Example A5: Preparation of compound No. A30



(A30)

(a) 0.176 g of LiH and 4.46 g of adenine are heated at 120°C for 1 h in 110 ml of DMF. After the mixture has cooled down to RT, a solution of 3.41 g of compound A1 in 3 ml of DMF is added and the whole is heated at 135°C for 6 h. The reaction mixture is then poured into a mixture composed of 300 ml of ethyl acetate and 100 ml of water; the aqueous phase is separated off and the organic phase is subsequently extracted a further 2 times with 100 ml of water on each occasion. After the organic phase has been dried over magnesium sulphate and the solvent has been evaporated off, the residue is purified by chromatography on silica gel using ethyl acetate/methanol/triethylamine, 8/2/0.01, as the eluent. 2.29 g of the compound A27 are obtained from the product-containing fractions by crystallization from ethyl acetate.

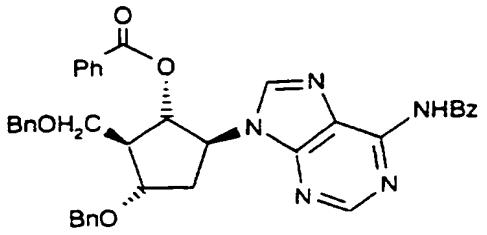


(A27)

^{1}H-NMR (250 MHz, DMSO-d_6, TMS): δ = 8.20 and 8.10 [2 x s, 2 x 1H, H-C(2) and H-C(8)]; 5.45 [d, 1H, HO-C(6')]; 4.75 [m, 1H]; 3.95 [m, 1H]; 3.60 [m, 2H, C(5')-H_2].

(b) A spatula tip of 4-(N,N-dimethylamino)pyridine is added to a solution of 0.800 g of compound A27 in 20 ml of pyridine, and 0.493 ml of benzoyl chloride is subsequently added dropwise to this solution at 0°C. The mixture is stirred at RT for 19 h with a further 0.220 ml

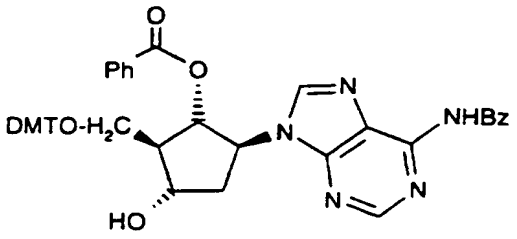
of benzoyl chloride being added after 18 h. 3 ml of water and 3 ml of conc. ammonia are then added at 0°C and the mixture is stirred at RT for 30 min. It is subsequently poured into 100 ml of ethyl acetate, and this mixture is extracted 3 x with 50 ml of water on each occasion; the organic phase is dried over magnesium sulphate and the solvent is removed on a rotary evaporator. The residue is dissolved in a mixture composed of ethyl acetate and methylene chloride and subsequently purified by chromatography on silica gel using ethyl acetate as the eluent. 1.02 g of compound A28 are obtained.



(A28)

^{1}H-NMR (250 MHz, $CDCl_3$, TMS): δ = 9.35 [s, 1H, NH]; 8.70 [s, 1H]; 8.10 [s, 1H]; 5.85 [m, 1H]; 5.45 [m, 1H]; 4.55 [s, 4H, CH_2, Bn]; 4.20 [m, 1H, H-C(3')]; 3.80 [m, 2H, C(5')-H_2].
MS: 654 ([M + H]$^+$).

(c) 10.8 ml of a 1 M solution of BCl_3 in methylene chloride are added, at -78°C, to a solution of 1.01 g of compound A28 in 50 ml of methylene chloride. After 3 h at -78°C, 10 ml of methanol are added and the mixture is concentrated by evaporation on a rotary evaporator and coevaporated a further 3 times with methanol. The residue is evaporated 3 times with pyridine and then dissolved in 20 ml of pyridine. 0.300 ml of triethylamine, 0.630 g of DMT chloride and a spatula tip of 4-(N,N-dimethylamino)pyridine are added to this solution, and the reaction mixture is stirred at RT for 20 h. It is then poured into 100 ml of ethyl acetate and the whole is extracted 3 times with 50 ml of water on each occasion; the organic phase is dried, the solvent is evaporated off and the residue is purified by chromatography on silica gel using ethyl acetate/triethylamine, 99/1, as the eluent. 0.416 g of compound A29 is obtained.



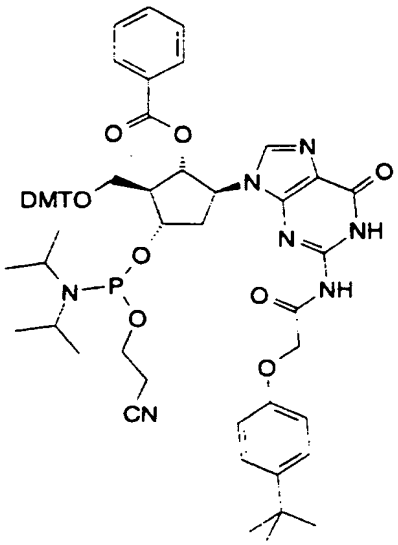
(A29)

^{1}H-NMR (250 MHz, $CDCl_3$, TMS): δ = 9.45 [s, 1H, NH]; 8.75 [s, 1H]; 8.05 [d, 2H, H-C(ar), Bz]; 7.95 [s, 1H]; 7.85 [d, 2H, H-C(ar), Bz]; 6.70 [m, 4H, H-C(ar), DMT]; 5.85 [m, 1H]; 5.20 [m, 1H]; 3.70 [d, 3H, OCH_3].

(d) Compound A30 is prepared in analogy with the preparation of compound A6.

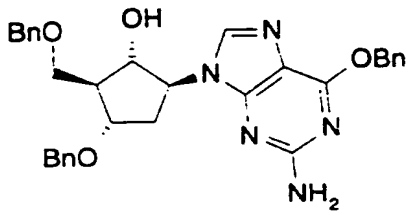
^{1}H-NMR (250 MHz, $CDCl_3$, TMS): δ = 9.15 [s, broad, 1H, NH]; 8.15 ["d", 1H]; 7.95 [m, 3H]; 7.85 [d, 2H]; 6.65 [m, 4H, H-C(ar), DMT]; 5.95 [m, 1H]; 5.15 [m, 1H].
^{31}P-NMR ($CDCl_3$): 148.291 and 148.206.

Example A6: Preparation of compound No. A36



(A36)

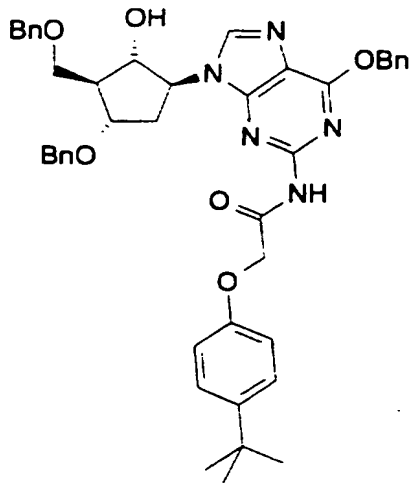
(a) 4.34 g of 2-amino-6-b nzyloxypurine (prepared in accordance with J. Kjellberg, M. Lilijenberg, *Tetrahedron Lett.* **1986**, *27*, 877) and 0.096 g of LiH are heated at 120°C for 1 h in 40 ml of DMF. After the reaction mixture has cooled down to 70°C, 1.86 g of compound A1 are added and the mixture is heated at 125°C for a further 4 h. The reaction mixture is then poured into 300 ml of ethyl acetate and the resulting solution is washed 3 times with 50 ml of water on each occasion; the organic phase is subsequently dried over $MgSO_4$. The residue which is obtained after concentrating the solution by evaporation is chromatographed 2 x on silica gel using ethyl acetate as the eluent on each occasion. 1.511 g of the compound A31 are obtained as a white foam.



(A31)

^{1}H-NMR (400 MHz, DMSO-d_6, TMS): δ = 8.05 [s, 1H, H-C(8)]; 7.6 -7.3 [m, 15H, H-C(ar)]; 6.45 [s, 2H, OC-H_2]; 5.55 [s, 2H, OC-H_2]; 5.50 [d, 1H, OH]; 4.7 [m, 1H, H-C(1')]; 4.6 [s, 2H,OC-H_2]; 4.55 [s, 2H, OC-H_2]; 4.2 [m, 1H, H-C(3')]; 3.7 [dd, 1H, H-C(5')]; 3.6 [dd, 1H, H-C(5')]; 2.35 - 2.15 [m, 3H, H-C(4') + 2 x H-C(2')].
MS: 552 [(M + H)$^+$].

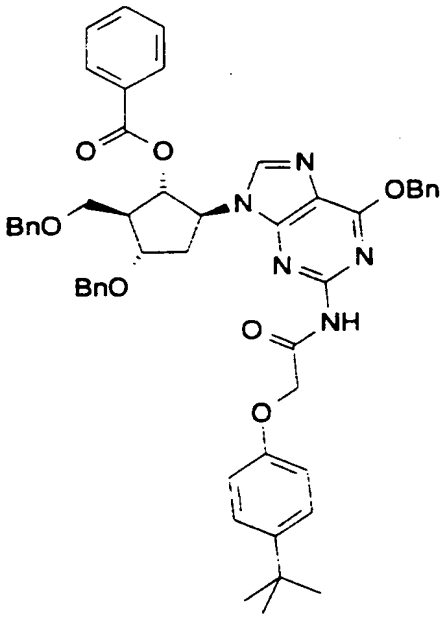
(b) 1.72 ml of $(CH_3)_3SiCl$ and a spatula tip of DMAP are added, at 0°C, to a solution of 1.50 g of compound A31 in 30 ml of abs. pyridine. The solution is then stirred at RT for 30 min. 3.25 g of tert-butylphenoxyacetic anhydride and 0.331 g of DMAP are subsequently added at 0°C. The reaction mixture is stirred at RT for 3 h and is then, after 1 ml of water has been added, poured into 200 ml of ethyl acetate. The solution is washed 3 x with 50 ml of water on each occasion and 1 x with 50 ml of a saturated solution of $NaHCO_3$, dried over $MgSO_4$ and concentrated by evaporation. The residue which remains is evaporated a further 3 x with toluene, then with 5 ml of acetic acid/water, 1/1, and then once again 3 x with toluene. Chromatography of the residue on silica gel using methylene chloride/ethyl acetate as the eluent yields 1.72 g of compound A32 as an amorphous, white solid.



(A32)

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 9.0 [s, br, 1H, NH]; 7.75 [s, 1H, H-C(8)]; 7.5 [d, 2H, H-C(ar)]; 7.4 - 7.2 [m, 15H, H-C(ar)]; 6.95 [d, 2H, H-C(ar)]; 5.6 [s, 2H, OC-H_2]; 4.75 [m, 1H, H-C(1')]; 4.35 [m, 1H, H-C(6')]; 4.05 [m, 1H, H-C(3')]; 3.75 [dd, 1H, H-C(5')]; 3.65 [dd, 1H, H-C(5')]; 2.7 [m, 1H, H-C(2')]; 2.6 [m, br, 1H, H-C(2')]; 2.3 [m, 1H, H-C(4')]; 1.3 [s, 9H, *tert.*-butyl].
MS: 741 [$(M)^+$].

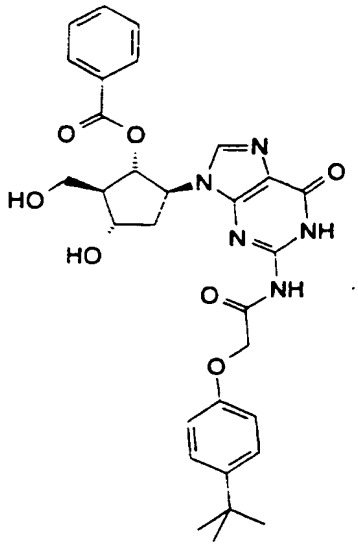
(c) 0.462 ml of pyridine 0.647 g of benzoic anhydride and 0.349 g of DMAP are added to a solution of 1.7 g of compound A32 in 30 ml of methylene chloride and the mixture is subsequently stirred at RT. After 3 h, a further 0.139 ml of pyridine, 0.194 g of benzoic anhydride and 0.104 g if DMAP are added. After a total of 18 h of stirring at RT, the reaction mixture is poured into a mixture composed of 200 ml of water and 50 ml of methylene chloride; the organic phase is separated off and the aqueous phase is extracted a further 2 x with 50 ml of methylene chloride on each occasion. The combined organic extracts are dried over $MgSO_4$ and then concentrated by evaporation. The residue is chromatographed on silica gel using methylene chloride/ether, 19/1 → 9/1, as the eluent. 1.85 g of the compound A33 are obtained as a white foam.



(A33)

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.95 [d, 2H, H-C(ar), Bz]; 7.85 [s, 1H, H-C(8)]; 7.55 [m, 3H, H-C(ar)]; 7.4 - 7.2 [m, 17H, H-C(ar)]; 6.95 [d, 2H, H-C(ar)]; 5.8 [t, 1H, H-C(6')]; 5.6 [s, 2H, OC-H_2]; 5.3 [m, 1H, H-C(1')]; 4.75 [s, 2H, OC-H_2]; 4.55 [m, 4H, OC-H_2]; 4.2 [m, 1H, H-C(3')]; 3.85 [m, 2H, C(5')-H_2]; 2.8 [m, 1H]; 2.65 [m, 1H]; 2.45 [m, 1H]; 1.3 [s, 9H, *tert*-butyl]. MS: 845 [$(M)^+$].

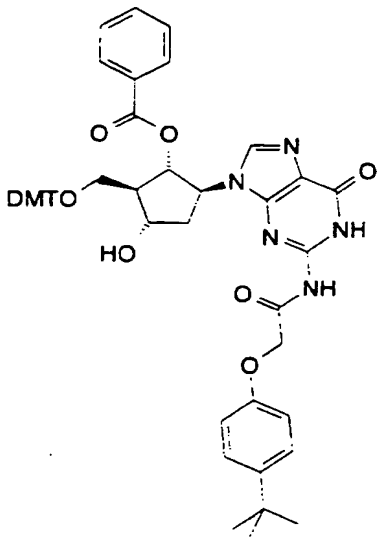
(d) 1.85 g of compound A33 are dissolved in 100 ml of methanol/tetrahydrofuran, 1/1, and are hydrogenated, in the presence of 2 N HCl, over Pd-C at atmospheric pressure and at RT. After 3.5 h, the catalyst is filtered off and the filtrate is then evaporated to dryness. The solid residue is recrystallized from hot chloroform/methanol with 0.492 g of the compound A34 being obtained as white crystals. A further 0.490 g of A34 is obtained from the mother liquor by evaporating and then titurating the residue with water.



(A34)

^{1}H-NMR (400 MHz, DMSO-d_6): δ = 11.7 [d, br, 2H, 2x NH]; 8.3 [s, 1H, H-C(8)]; 8.0 [d, 2H, H-C(ar), Bz]; 7.85 [t, 1H, H-C(ar), Bz]; 7.6 [t, 2H, H-C(ar), Bz]; 7.4 [d, 2H, H-C(ar)]; 6.95 [d, 2H, H-C(ar)]; 5.55 [m, 1H, H-C(6')]; 5.35 [m, 1H, H-C(1')]; 4.9 [d, 2H, OC-H_2]; 4.35 [m, 1H, H-C(3')]; 3.75 [m, 2H, C(5')-H_2]; 2.65 [m, 1H]; 2.3 - 2.2 [m, 2H]; 1.3 [s, 9H, *tert*-butyl].

(e) The compound A35 is prepared in analogy with the preparation of compound A5.

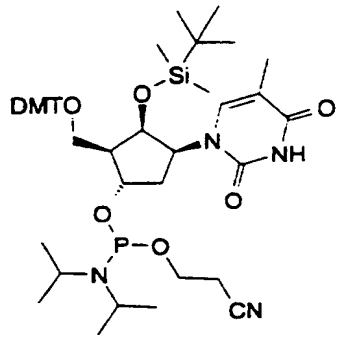


(A35)

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.9 [d, 2H, H-C(ar), Bz]; 7.7 [s, 1H, H-C(8)]; 7.5 - 7.1 [m, 14H, H-C(ar)]; 7.75 - 7.65 [m, 6H, H-C(ar)]; 7.4 [d, 2H, H-C(ar)]; 5.85 [t, 1H, H-C(6')]; 5.15 [q, 1H, H-C(1')]; 4.55 [s, 2H, OC-H_2]; 4.45 [m, 1H, H-C(3')]; 3.7 [s, 6H, OCH_3]; 3.45 [m, 2H, C(5')-H_2]; 2.6 [m, 1H]; 2.45 - 2.35 [m, 2H]; 1.3 [s, 9H, *tert*-butyl].

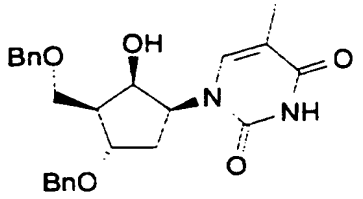
(f) The compound A36 is prepared in analogy with the preparation of compound A6.

Example A7: Preparation of compound No. A41.



(A41)

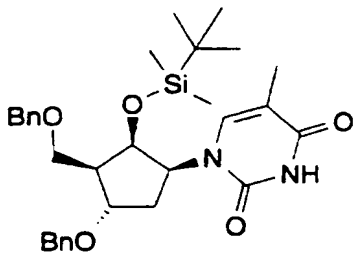
(a) 3 ml of 1 N NaOH are added to a solution of 0.418 g of compound A19 in 12 ml of MeOH and the mixture is stirred at RT for 2 h and then at 40°C for 18 h. After it has cooled down to RT, the mixture is poured into 100 ml of ethyl acetate and the solution is washed 3 x with 50 ml of ice water on each occasion. The combined aqueous extracts are back-extracted with ethyl acetate/THF, 4/1, and the various organic extracts are subsequently concentrated by evaporation. The evaporation residues are chromatographed jointly on silica gel using ethyl acetate as the eluent. 0.390 g of the compound A37 is obtained as a white foam.



(A37)

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 8.35 [s, br, 1H, NH]; 7.4 - 7.2 [m, 11H, H-C(ar) + H-C(6)]; 5.1 [m, 1H, H-C(1')]; 4.6 - 4.4 [m, 5H, 2x OC-H_2 + H-C(6')]; 4.2 [m, 1H, H-C(3')]; 3.8 [m, 2H, C(5')-H_2]; 3.35 [s, 1H, OH]; 2.3 [m, 2H]; 2.2 [m, 1H]; 1.9 [s, 3H, C(5)-CH_3].
MS: 436 [$(M)^+$].

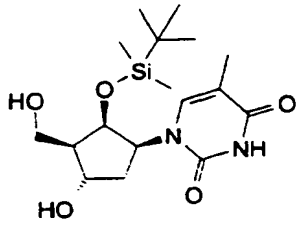
(b) 1.07 g of TBDMS-Cl and 0.602 g of imidazole are added to a solution of 2.57 g of compound A37 in 30 ml of DMF. After the mixture has been stirred at RT for 6 h, 0.06 g of DMAP is added in addition. The mixture is allowed to react at RT for a further 12 h. It is then heated at 60°C for 6 h, then at 90°C for 2 h, at 110°C for 16 h and finally at 130°C for 4 h. After it has been cooled down to RT, the solution is poured into a mixture composed of 100 ml of ethyl acetate and 50 ml of water, and the organic phase is separated off, washed a further 2 x with 50 ml of water on each occasion, then dried over $MgSO_4$ and concentrated by evaporation. The residue is purified by chromatography on silica gel using methylene chloride/ether, 4/1, (+ 1% triethylamine) as the eluent. 2.82 g of the compound A38 are obtained as a white foam.



(A38)

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 8.4 [s, br, 1H, NH]; 7.3 [m, 10H, H-C(ar)]; 7.1 [s, 1H, H-C(6)]; 5.05 [m, 1H, H-C(1')]; 4.6 - 4.4 [m, 5H, 2x OC-H_2 + H-C(6')]; 3.9 [m, 1H, H-C(3')]; 3.6 [dd, 1H, H-C(5')]; 3.45 [dd, 1H, H-C(5')]; 2.5 [m, 1H]; 2,3 [m, 1H]; 2.05 [m, 1H]; 1.85 [s, 3H, C(5)-CH_3]; 0.8 [s, 9H, *tert*-butyl].
MS: 551 [$(M+H)^+$].

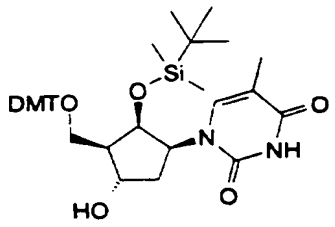
(c) 1.10 g of compound A38 are hydrogenated over 0.2 g of 10 % Pd-C, at atmospheric pressure and at RT, for 3 h in 20 ml of THF/methanol, 1/1. 0.768 g of the compound A39 is obtained as a white crystalline solid after filtering off the catalyst and concentrating the filtrate by evaporation.



(A39)

^{1}H-NMR (400 MHz, MeOH-d_4): δ = 7.3 [s, 1H, H-C(6)]; 4.95 [m, 1H, H-C(1')]; 4.35 [m, H-C(6')]; 4.05 [m, 1H, H-C(3')]; 3.65 [m, 2H, C(5')-H_2]; 2.4 [m, 1H]; 2.0 [m, 1H]; 1.8 [m, 4H, (incl. s, C(5)-CH_3)]; 0.8 [s, 9H, *tert*-butyl].
MS: 313 [(M-*tert*-butyl)$^+$].

(d) The compound A40 is prepared in analogy with the preparation of compound A5.



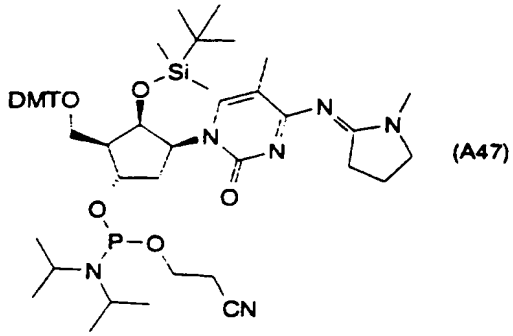
(A40)

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 8.45 [s, 1H, NH]; 7.05 [s, 1H, H-C(6)]; 6.95 [d, 4H, H-C(ar)]; 5.2 [m, 1H, H-C(1')]; 4.45 [m, H-C(6')]; 4.2 [t, 1H, H-C(3')]; 3.45 [dd, 1H, H-C(5')]; 3.15 [dd, 1H, H-C(5')]; 2.3 [m, 2H]; 2.1 [m, 1H]; 1.85 [s, 3H, C(5)-CH_3]; 0.8 [s, 9H, *tert*-butyl].
MS: 672 [(M)$^+$].

(e) The compound A41 is prepared in analogy with the preparation of compound A6 (2 diastereoisomers).

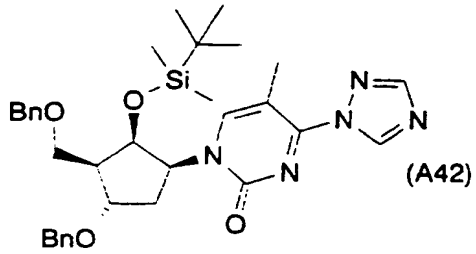
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 8.25 [s, 1H, NH]; 7.05, 6.95 [2x s, each 0,5H, H-C(6)]; 6.8 [d, 4H, H-C(ar)]; 5.15 [m, 1H, H-C(1')]; 4.45 [m, H-C(6')]; 4.2 [t, 1H, H-C(3')]; 3.8 ["d", 6H, OCH_3 (DMT)]; 1.85 [s, 3H, C(5)-CH_3]; 0.8 [s, 9H, *tert*-butyl].
^{31}P-NMR ($CDCl_3$): 148.184 and 147.553.

Example 8: Preparation f compound No. A47



(A47)

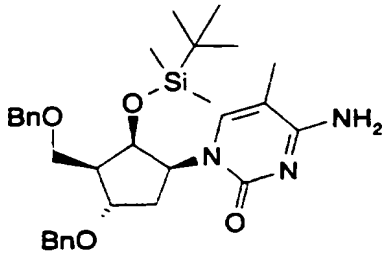
(a) The compound A42 is prepared proceeding from compound A38 in analogy with the preparation of compound A13.



(A42)

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 9.3 [s, 1H]; 8.1 [s, 1H]; 7.65 [s, 1H, H-C(6)]; 7.3 [m, 10H, H-C(ar)]; 5.35 [m, 1H, H-C(1')]; 4.65 [t, 1H, H-C(6')]; 4.6 - 4.4 [m, 4H, 2x OC-H_2]; 3.95 [m, 1H, H-C(3')]; 3.6 [dd, 1H, H-C(5')]; 3.5 [dd, 1H, H-C(5')]; 2.6 [m, 1H]; 2.4 [s, 3H, C(5)-CH_3]; 2.35 [m, 1H]; 2.1 [m, 1H]; 0.8 [s, 9H, *tert*-butyl].
MS: 544 [(M-*tert*-butyl)$^+$].

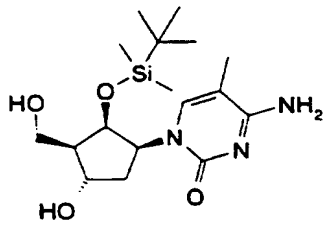
(b) The compound A43 is prepared in analogy with the preparation of compound A14.



(A43)

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.3 [m, 10H, H-C(ar)]; 7.1 [s, 1H, H-C(6)]; 5.25 [m, 1H, H-C(1')]; 4.6 - 4.4 [m, 5H, 2x OC-H_2 + H-C(6')]; 3.9 [m, 1H, H-C(3')]; 3.6 [dd, 1H, H-C(5')]; 3.45 [dd, 1H, H-C(5')]; 2.5 [m, 1H]; 2.25 [m, 1H]; 2.0 [m, 1H]; 1.85 [s, 3H, C(5)-CH_3]; 0.8 [s, 9H, *tert*-butyl].
MS: 544 [(M-*tert*-butyl)$^+$].

(c) 1.1 g of compound A43 are hydrogenated over 0.2 g of 10 % Pd-C, at atmospheric pressure and at RT, for 1.5 h in methanol/THF, 1/1, and in the presence of conc. HCl. After the addition of saturated, aqueous $NaHCO_3$ solution, the catalyst and additional undissolved material are filtered off. The filtrate is concentrated by evaporation, the residue is suspended in THF/methanol 4/1, and the product is filtered off. 0.907 g of the compound A44 is obtained as a white foam.

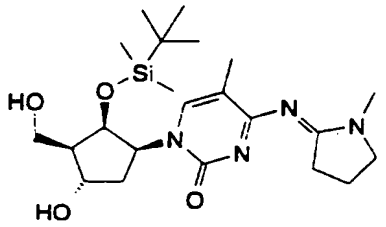


(A44)

^{1}H-NMR (400 MHz, DMSO-d_6): δ = 7.3 [s, 1H, H-C(6)]; 7.0 [s, br, 1H]; 6.6 [s, br, 1H]; 4.95 [m, 1H, H-C(1')]; 4.8 [s, br, 1H, OH]; 4.35 [s, br, 1H, OH]; 4.25 [m, 1H, H-C(6')]; 3.9 [m, 1H, H-C(3')]; 3.5 [m, 2H, C(5')-H_2]; 2.35 [m, 1H]; 1.95 [m, 1H]; 1.8 [s, 3H, C(5)-CH_3]; 1.65 [m, 1H]; 0.8 [s, 9H, *tert*-butyl].
MS: 370 [(M+H)$^+$].

(d) 1.032 g of N-methylpyrrolidine dimethyl acetal are added to a solution of 0.89 g of compound A44 in 20 ml of methanol and the mixture is allowed to react at RT for 18 h. 0.5 ml of water are then added and then, after a further 15 min, the reaction mixture is concentrated by evaporation. The residue is purified by chromatography on silica gel using

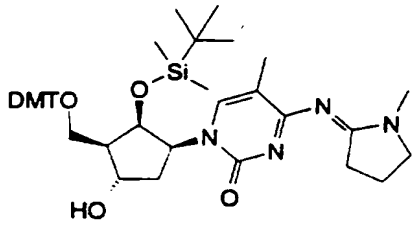
chloroform/methanol, 4/1, (+ 1 % triethylamine) as the eluent. 0.948 g of th compound A45 is obtained as a white foam.



(A45)

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.2 [s, 1H, H-C(6)]; 5.35 [m, 1H, H-C(1')]; 4.5 [m, 2H, H-C(3') + H-C(6')]; 3.85 [m, 2H]; 3.15 [m, 1H]; 3.05 [s, 3H]; 3.0 [m, 1H]; 2.4 [m, 2H]; 2.25 [m, 1H]; 2.05 [m, 3H]; 1.95 [s, 3H, C(5)-CH_3]; 0.8 [s, 9H, *tert*-butyl].
MS: 450 [$(M)^+$].

(e) The compound A46 is prepared in analogy with the preparation of compound A5.



(A46)

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.4 - 7.2 [m, 9H]; 7.15 [s, 1H, H-C(6)]; 6.8 [m, 4H]; 5.4 [m, 1H, H-C(1')]; 4.45 [m, 1H, H-C(3')]; 4.3 [m, 1H, H-C(6')]; 3.8 [m, 6H]; 3.4 [m, 3H]; 3.2 - 3.05 [m, 2H]; 3.0 [s, 3H]; 2.35 [m, 2H]; 3.15 [m, 1H]; 3.05 [s, 3H]; 2.05 [m, 3H]; 1.9 [s, 3H, C(5)-CH_3]; 0.6 [s, 9H, *tert*-butyl].

(f) The compound A47 is prepared in analogy with the preparation of compound A6.

B) Preparation of oligonucleotides

Oligonucleotides are prepared on a solid support (controlled pore glass, CPG) with a DNA synthesizer (Applied Biosystems, Model 380 B, Standard phosphoramidite chemistry and oxidation with iodine) using the novel dimethoxytritylated and 3'-activated [3'-(β-cyanoethoxydi(i-propylamino)phosphoramidite)] nucleosides or natural nucleosides which

are activated in this manner. The synthesis is carried out in accordanc with the manufacturer's standard protocols [cf., also "Oligonucleotide Synthesis: A Practical Approach" M.J. Gait; IRL Press 1984 (Oxford and Washington DC)]. After the last nucleoside building block has been coupled on, the 5'-protected oligonucleotide is released from the support, in conjunction with the removal of all the remaining protecting groups, by treating with concentrated aqueous ammonia overnight, and subsequently purified by reverse-phase HPLC using 50 mM ammonium acetate buffer (pH 7)/acetonitrile. The 5'-dimethoxytrityl protecting group is subsequently removed by a 20-minute treatment with 80 % aqueous acetic acid, after which the oligonucleotide is precipitated with ethanol and isolated by centrifugation. The purity of the oligonucleotide is examined by gel electrophoresis (polyacrylamide) and its identify is tested by means of matrix-assisted laser desorption time-of-flight mass spectrometry (MALDI-TOF MS).

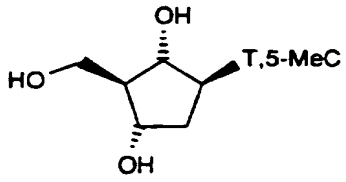
C) Binding behaviour and stability

Example C1: Affinity; interaction of the (antisense) oligonucleotides with complementary (sense) oligoribonucleotide sequences

The interaction of the oligonucleotides with the corresponding base-complementary oligomers of the natural ribonucleotides is elucidated by plotting UV melting curves and determining the T_m values from these curves. This standard method is described, for example, by Marky, L.A., Breslauer, K.J., Biopolymers 26:1601-1620 (1987). Solutions of the oligonucleotides and the corresponding base-complementary natural oligoribonucleotides is prepared in 10 mM phosphate buffer, 100 mM NaCl, 0.1 mM EDTA, pH = 7.0 ($c = 4.10^{-6}$ M/oligonucleotide), and the change in extinction at 260 nm is plotted against the temperature (15 to 95°C). The respective T_m value is determined from the melting curves which are obtained (Table 1).

Table 1: Affinity of modified oligonucleotides vs. RNA

t, c =



Oligonucleotide	SEQU. ID. NO.	Tm(°C)	ΔTm(°C)
GCGTTTTTTTTTGCG	1	48.0	-
GCGttttttttGCG	2	56.0	8.0
TTTTTCTCTCTCTCT	3	52.3	-
tttttctctctctcT	4	63.4	11.1
		57.9**	5.6**
CTCGTACTTTTCCGGTCC	5	61.7	-
CTCGTACttttCCGGTCC	6	62.5	0.8
TTCTCGCCCGCTCCTCCTCC*	7	68.6	-
ttctcGCCCGCTCCtcctcC*	8	72.4	3.8

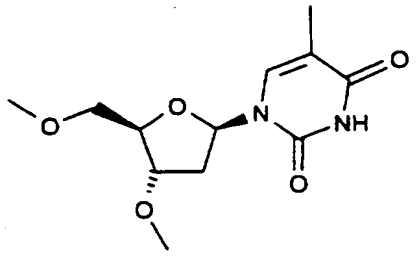
*All the inter-nucleoside bridging groups are -P(O)S$^-$-

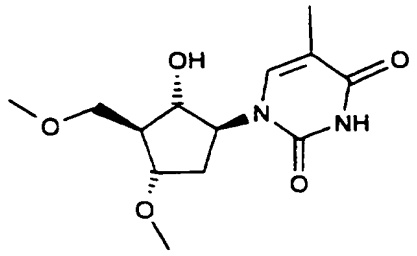
** Corrected for the influence of 5-MeC

Example C2: Nuclease stability; Enzymic hydrolysis of different oligonucleotides having the sequence d(TCC AGG TGT CCG ttt C) (SEQ. ID. NOs. 9 and 10, see below)

In each case, 14 µg of the synthetic oligonucleotide or of the corresponding natural oligomer are incubated at 37°C in 200 µl of 10 % heat-inactivated foetal calf serum (c =

70 µg/ml). After 0.5, 1, 2, 4, 6, 24 and 48 hours, 15 µl of the reaction solution are in ach case quenched by being added to 25 µl of 9 M urea and tris-borate buffer (pH 7), with these mixtures then being stored at -20°C until measured. The quenched reaction solutions are fractionated by polyacrylamide gel electrophoresis and the cleavage products are determined by way of their phosphorus content (phosphoimager method). The ratio R of the sum of the concentrations of the completely intact oligonucleotide ($c_n^{(t)}$) and of the fragment ($c_{n-1}^{(t)}$) resulting from the elimination of the natural C building block from the 3' end, at a given time t, to the starting concentration of the completely intact oligonucleotide at time t = 0 ($c_n^{(0)}$), $R = (c_n^{(t)} + c_{n-1}^{(t)})/c_n^{(0)}$, is plotted against time. The half-life lives, $\tau_{1/2}$, which are determined in this way, that is the times for which R = 0.5, are

for t =  (SEQ. ID. NO. 9) $\tau_{1/2}$ = 2.0 h,

for t =  (SEQ. ID. NO. 10) $\tau_{1/2}$ = 14 h.

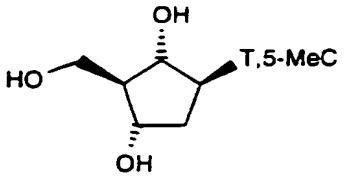
D) Biological activity

Example D: Inhibition of the expression of c-raf kinase

TTCTCGCCCGCTCCTCCTCC*: Oligonucleotide I (SEQ. ID. NO. 7)

ttctcGCCCGCTCCtcctcC*: Oligonucleotide II (SEQ. ID. NO. 8)

*All the inter-nucleoside bridging groups are $-P(O)S^-$;

t, c = 

T-24 cells are treated with oligonucleotide I or oligonucleotide II in serum-free Optimem Medium, which contains 10 µg/ml Lipofectin. (The oligonucleotides are added directly to the medium). Following a 4-hour incubation at 37°C, the oligonucleotide-containing medium is removed and replaced by oligonucleotide-free, normal medium (McCoy's medium + 10 % FCS). After a total of 24 h, the RNA of the cells is extracted and purified by the guanidinium isothiocyanate method. The expression of c-raf RNA is subsequently analyzed using a radioactively labelled human c-DNA probe. The c-raf RNA is quantified using a phospho-imager.

In the case of oligonucleotide I, the IC_{50} for inhibiting the synthesis of c-raf kinase mRNA is approximately 300 nM, while it is approximately 40 nM in the case of oligonucleotide II.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: CIBA-GEIGY AG
(B) STREET: Klybeckstr. 141
(C) CITY: Basel
(E) COUNTRY: Switzerland
(F) POSTAL CODE (ZIP): 4002
(G) TELEPHONE: +41 61 69 11 11
(H) TELEFAX: + 41 61 696 79 76
(I) TELEX: 962 991

(ii) TITLE OF INVENTION: 6'-Substituted carbocyclic nucleosides

(iii) NUMBER OF SEQUENCES: 10

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: Patentin Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GCGTTTTTTT TTTGCG 16

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(iv) ANTI-SENSE: YES

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION:4..13
(D) OTHER INFORMATION:/note= "modified sugar"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

GCGTTTTTTT TTTGCG 16

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

TTTTTCTCTC TCTCT 15

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(iv) ANTI-SENSE: YES

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION:1..14
(D) OTHER INFORMATION:/note= "modified sugar"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

TTTTTCTCTC TCTCT 15

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

CTCGTACTTT TCCGGTCC 18

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(iv) ANTI-SENSE: YES

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION:8..11
(D) OTHER INFORMATION:/note= "modified sugar"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

CTCGTACTTT TCCGGTCC 18

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(iv) ANTI-SENSE: YES

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION:1..20
(D) OTHER INFORMATION:/note= "modified backbone"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

TTCTCGCCCG CTCCTCCTCC 20

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(iv) ANTI-SENSE: YES

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION:1..20
(D) OTHER INFORMATION:/note= "modified backbone"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION:1..5
(D) OTHER INFORMATION:/note= "modified sugar"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION:15..19
(D) OTHER INFORMATION:/note= "modified sugar"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

TTCTCGCCCG CTCCTCCTCC 20

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

TCCAGGTGTC CGTTTC 16

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(iv) ANTI-SENSE: YES

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION:13..15
(D) OTHER INFORMATION:/note= "modified sugar"

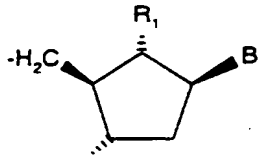
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

TCCAGGTGTC CGTTTC 16

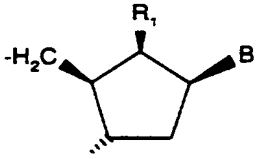
Patent claims

What is claimed is

1. An oligonucleotide which contains from 2 to 200 identical or different residues of natural or synthetic nucleosides which are linked via a nucleotide-bridging group Y, wherein at least two of the nucleosides are, independently of each other, a residue of the formula Ia or Ib



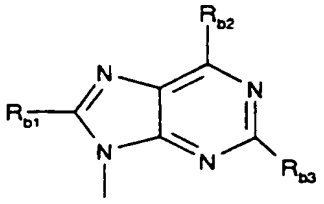
(Ia)



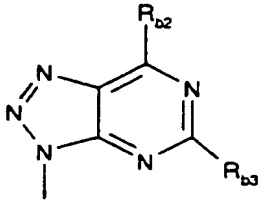
(Ib)

in which R_1 is OH or NH_2 and B is a pyrimidine or purine residue, or an analogue thereof, and at least two of these nucleosides are consecutive on at least one occasion.

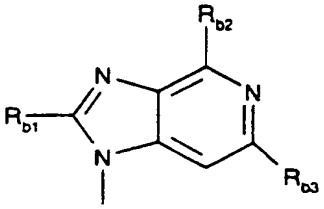
2. An oligonucleotide according to claim 1, wherein B, as a purine residue or an analogue thereof, is a residue of the formula II, IIa, IIb, IIc, IId, IIe or IIf



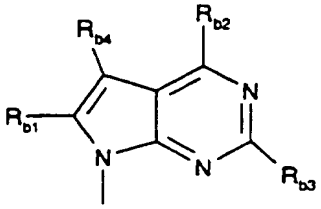
(II),



(IIa),



(IIb),



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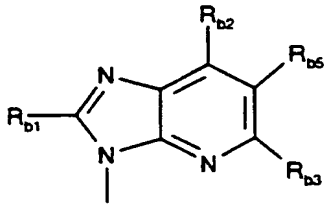
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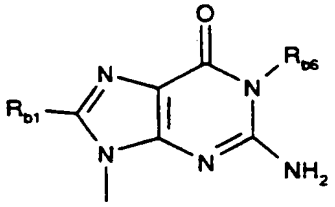
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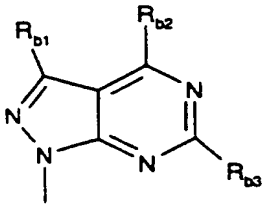

(IIf),

in which R_{b1} is H, Cl, Br, OH or -O-C_1-C_{12}alkyl, and R_{b2}, R_{b3} and R_{b5} are, independently of each other H, OH, SH, NH_2, $NHNH_2$, NHOH, NHO-C_1-C_{12}alkyl, -N=CH-N(C_1-C_{12}alkyl)$_2$, -N=CH-Ncycloalkyl, F, Cl, Br, C_1-C_{12}alkyl, hydroxy-C_1-C_{12}alkyl, amino-C_1-C_{12}alkyl, C_1-C_{12}alkoxy, benzyloxy, C_1-C_{12}alkylthio, where the hydroxyl and amino groups are unsubstituted or substituted by a protecting group, phenyl, benzyl, primary amino having from 1 to 20 C atoms, or secondary amino having from 2 to 30 C atoms, R_{b4} is hydrogen, CN or -C≡C-R_{b7}, R_{b6} and R_{b7} are hydrogen or C_1-C_4alkyl.

3. An oligonucleotide according to claim 2, wherein the primary amino contains from 1 to 12 C atoms and the secondary amino contains from 2 to 12 C-Atoms.

4. An oligonucleotide according to claim 3, wherein the primary amino and the secondary amino are radicals of the formula $R_{a1}R_{a2}N$, in which R_{a1} is H or, independently, has the meaning of R_{a2}, and R_{a2} is C_1-C_{20}-alkyl, -aminoalkyl or -hydroxyalkyl; carboxyalkyl or carbalkoxyalkyl wherein the carbalkoxy group contains from 2 to 8 C atoms and the alkyl group contains from 1 to 6 C atoms; C_2-C_{20}-alkenyl; phenyl, mono- or di-(C_1-C_4-alkyl- or -alkoxy)phenyl, benzyl, mono- or di-(C_1-C_4-alkyl- or -alkoxy)benzyl; or 1,2-, 1,3- or 1,4-imidazolyl-C_1-C_6alkyl, or R_{a1} and R_{a2} are, together, tetra- or penta-methylene, 3-oxa-1,5-pentylene, -CH_2-NR_{a3}-CH_2CH_2- or -CH_2CH_2-NR_{a3}-CH_2CH_2-, in which R_{a3} is H or C_1-C_4alkyl.

wherein the amino group in th aminoalkyl is unsubstituted or substituted by one or two C_1-C_4-alkyl or -hydroxyalkyl groups, and the hydroxyl group in the hydroxyalkyl is free or esterified with C_1-C_4alkyl.

5. An oligonucleotide according to claim 4, wherein the primary amino and the secondary amino are methyl-, ethyl-, dimethyl-, diethyl-, allyl-, mono- or di(hydroxyeth-2-yl)-, phenyl-, benzyl-, acetyl-, isobutyryl- or benzoylamino.

6. An oligonucleotide according to claim 2, wherein R_{b1} in the formulae II, IIb, IIc, IId and IIe is hydrogen.

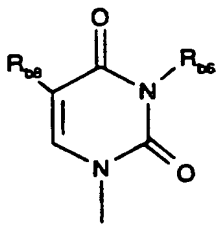
7. An oligonucleotide according to claim 2, wherein R_{b5} in the formula IId is hydrogen.

8. An oligonucleotide according to claim 2, wherein R_{b2} and R_{b3} in the formulae II, IIb, IIc, IId and IIe are, independently of each other, H, F, Cl, Br, OH, SH, NH_2, NHOH, $NHNH_2$, methylamino, dimethylamino, benzoylamino, methoxy, ethoxy or methylthio.

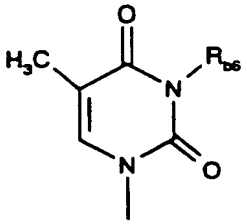
9. An oligonucleotide according to claim 2, wherein B is a purine residue or a residue of a purine analogue selected from the group consisting of adenine, N-methyladenine, N-benzoyladenine, 2-methylthioadenine, 2-aminoadenine, 6-hydroxypurine, 2-amino-6-chloropurine, 2-amino-6-methylthiopurine, guanine and N-isobutyrylguanine.

10. An oligonucleotide according to claim 9, wherein B is a purine residue or a residue of a purine analogue selected from the group consisting of adenin, 2-aminoadenine and guanine.

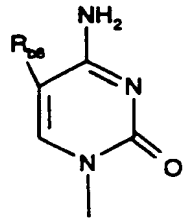
11. An oligonucleotide according to claim 1, wherein B is a uracil, thymine or cytosine residue of the formula III, IIIa, IIIb or IIIc



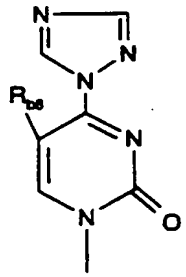
(III),



(IIIa)



(IIIb),



(IIIc)

in which R_{b6} is H or C_1-C_4alkyl, and R_{b8} is H, OH, SH, NH_2, $NHNH_2$, NHOH, NHO-C_1-C_{12}-alkyl, -N=CH-N(C_1-C_{12}alkyl)$_2$, -N=CH-Ncycloalkyl, F, Cl, Br, C_1-C_{12}alkyl, hydroxy-C_1-C_{12}-alkyl, amino-C_1-C_{12}alkyl, C_1-C_{12}alkoxy, benzyloxy, C_1-C_{12}alkylthio, where the hydroxyl and amino groups are unsubstituted or substituted by a protecting group, phenyl, benzyl, primary amino having from 1 to 20 C atoms, secondary amino having from 2 to 30 C atoms, C_1-C_{12}-alkenyl or C_1-C_{12}alkynyl, and the NH_2 group in formula IIIb is unsubstituted or substituted by C_1-C_6alkyl, benzoyl or a protecting group, and also the dihydro derivatives of the residues of the formulae III, IIIa, IIIb and IIIc.

12. An oligonucleotide according to claim 11, wherein R_{b8} is H, C_1-C_6-alkyl or -alkoxy or -hydroxyalkyl, C_2-C_6-alkenyl or -alkynyl, F, Cl, Br, NH_2, benzoylamino or mono- or di-C_1-C_6-alkylamino.

13. An oligonucleotide according to claim 12, wherein R_{b8} is H, F, Cl, Br, NH_2, $NHCH_3$, $N(CH_3)_2$, C_1-C_4alkyl, C_2-C_4alkenyl-(1) or C_2-C_4alkynyl-(1).

14. An oligonucleotide according to claim 13, wherein R_{b8} is H, C_1-C_4alkyl, C_2-C_4alkenyl-(1), C_2-C_4alkynyl-(1), NH_2, $NHCH_3$, $N(CH_3)_2$ or $(CH_3)_2N$.

15. An oligonucleotide according to claim 11, wherein B, as the residue of a pyrimidine analogue, is derived from uracil, thymine, cytosine, 5-fluorouracil, 5-chlorouracil, 5-bromouracil, dihydrouracil, 5-methylcytosine, 5-propynyluracil or 5-propynylcytosine.

16. An oligonucleotide according to claim 15, wherein B, as the residue of a pyrimidine analogue, is derived from uracil, thymine, cytosine, 5-methylcytosine, 5-propynyluracil or 5-propynylcytosine.

17. An oligonucleotide according to claim 1, wherein the bridging group Y is $-P(O)O^-$-, $-P(O)S^-$-, $-P(S)S^-$-, $-P(O)R_2$-, $-P(O)NR_3R_4$-, or $-CH_2$-, in which R_2 is H or C_1-C_6alkyl, and R_3 and R_4, independently of each other, have the meaning of R_2.

18. An oligonucleotide according to claim 17, wherein the bridging group Y is $-P(O)O^-$-.

19. An oligonucleotide according to claim 17, wherein the bridging group Y is $-P(O)S^-$-.

20. An oligonucleotide according to claim 17, wherein the bridging group Y within the same oligonucleotide at each position is independently $-P(O)O^-$- or $-P(O)S^-$-.

21. An oligonucleotide according to claim 1, which contains from 2 to 100 nucleoside residues.

22. An oligonucleotide according to claim 21, which contains from 3 to 50 nucleoside residues.

23. An oligonucleotide according to claim 22, which contains from 5 to 29 nucleoside residues.

24. An oligonucleotide according to claim 1, wherein the residues of the formulae Ia and/or Ib are bonded terminally and/or within the nucleotide sequence, with all or several, however at least 2, of the residues of the formulae Ia and/or Ib being consecutive.

25. An oligonucleotide according to claim 1, wherein from 2 to all the nucleotide residues are selected from the group consisting of residues of the formula Ia and Ib.

26. An oligonucleotide according to claim 25, wherein the residues of the formula Ia or Ib are either all consecutive or are distributed over the sequence, with it being necessary for 2 such residues to be consecutive on at least one occasion.

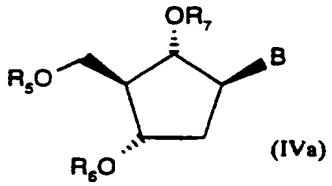
27. An oligonucleotide according to claim 1, wherein at least two nucleoside residues of the formula Ia in which $R_1 = NH_2$ are present.

28. An oligonucleotide according to claim 1, wherein at least two nucleoside residues of the formula Ia in which $R_1 = OH$ are present.

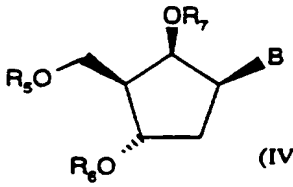
29. An oligonucleotide according to claim 1, wherein at least two nucleoside residues of the formula Ib in which $R_1 = NH_2$ are present.

30. An oligonucleotide according to claim 1, wherein at least two nucleoside residues of the formula Ib in which $R_1 = OH$ are present.

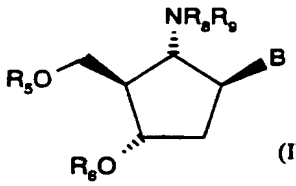
31. A process for preparing oligonucleotides according to claim 1, which comprises introducing at least two derivatives of the formula IVa, IVb, IVc or IVd



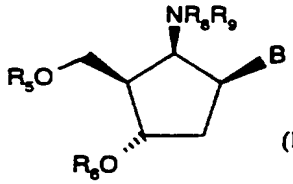
(IVa)



(IVb)



(IVc)



(IVd)

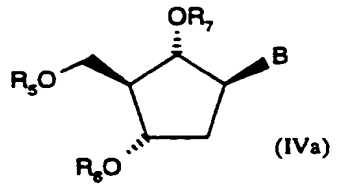
in which R_5 and R_7 are, independently of each other, a protecting group, R_8 and R_9 are, independently of each other, H or a protecting group, or R_8 and R_9 are bonded together in a single protecting group, B has one of the meanings given above for the residues of the formulae Ia and Ib, and R_6 is a residue which forms a phosphorus-containing nucleotide-bridging group, into the synthesis.

32. The use of an oligonucleotide according to claim 1 as a diagnostic agent for detecting viral infections or genetically determined diseases.

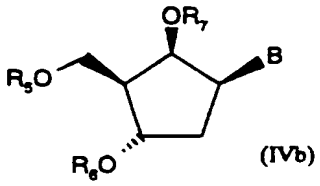
33. An oligonucleotide according to claim 1 for use in a therapeutic process for treating diseases in warm-blooded animals including man.

34. A pharmaceutical preparation which comprises an effective amount of an oligonucleotide according to claim 1, on its own or together with other active compounds, a pharmaceutical excipient material and, if desired, auxiliary substances.

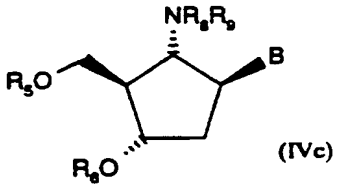
35. A compound of the formula IVa, IVb, IVc or IVd



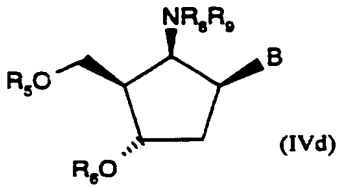
(IVa)



(IVb)



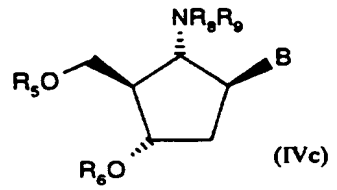
(IVc)



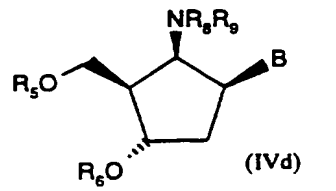
(IVd)

in which R_5 and R_7 are, independently of each other, a protecting group, R_8 and R_9 are, independently of each other, H or a protecting group, or R_8 and R_9 are bonded together in a single protecting group, B has one of the meanings given according to claim 2 for the residues of the formulae Ia and Ib, and R_6 is a radical which forms a phosphorus-containing nucleotide-bridging group.

36. A compound of the formula IVc or IVd



(IVc)

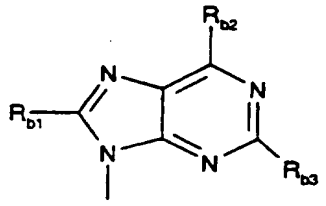


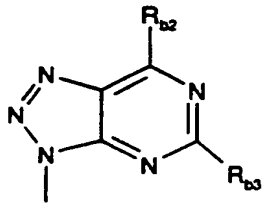
(IVd)

in which R_5 and R_7 are, independently of each other, a protecting group, R_8 and R_9 are, independently of each other, H or a protecting group, or R_8 and R_9 are bonded together in a single protecting group, B has one of the meanings according to claim 2 for the residues of the formulae Ia and Ib, and R_6 is a radical which forms a phosphorus-containing nucleotide-bridging group.

37. A compound according to claim 35, wherein R_5, R_7, R_8 and R_9 are, as protecting groups, benzyl, methylbenzyl, dimethylbenzyl, methoxybenzyl, dimethoxybenzyl, halogenated benzyl, in particular bromobenzyl; diphenylmethyl, di(methylphenyl)methyl, di(dimethylphenyl)methyl, di(methoxyphenyl)methyl, di(methoxyphenyl)(phenyl)methyl, triphenylmethyl, tris-4,4',4''-tert-butylphenylmethyl, di-p-anisylphenylmethyl, tri(methylphenyl)methyl, tri(dimethylphenyl)methyl, tri(methoxyphenyl)methyl, tri(dimethoxyphenyl)methyl; trimethylsilyl, triethylsilyl, tri-n-propylsilyl, i-propyl-dimethylsilyl, t-butyldimethylsilyl, t-butyldiphenylsilyl, n-octyldimethylsilyl, (1,1,2,2-tetramethylethyl)dimethylsilyl, $-(CH_3)_2Si-O-Si(CH_3)_2-$, $-(i-C_3H_7)_2Si-O-Si(i-C_3H_7)_2-$; acetyl, propanoyl, butanoyl, pentanoyl, hexanoyl, benzoyl, methylbenzoyl, methoxybenzoyl, chlorobenzoyl and bromobenzoyl; methyl-, ethyl-, propyl-, butyl-, phenyl-, benzyl-, p-bromo-, p-methoxy- and p-methylphenylsulphonyl; methoxy-, ethoxy-, n- or i-propoxy- or n-, i- or t-butoxycarbonyl, or phenyloxycarbonyl, benzyloxycarbonyl, methyl- or methoxy- or chlorophenyloxycarbonyl or -benzyloxycarbonyl or 9-fluorenylmethyloxycarbonyl.

38. A compound according to claim 35, wherein B, as a purine residue or an analogue thereof, is a residue of the formula II, IIa, IIb, IIc, IId, IIe or IIf


(II),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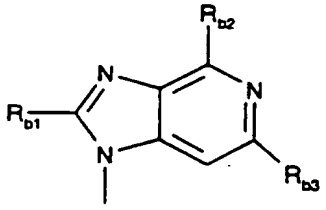
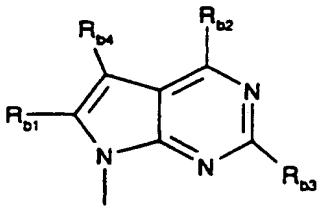

(IIb),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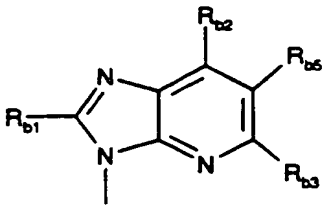
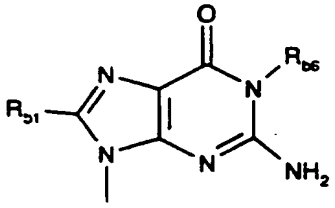
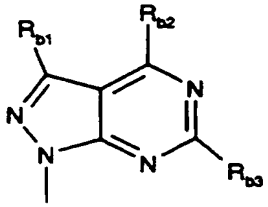


(IId),



(IIe),



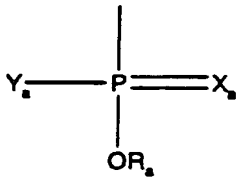
(IIf),

in which R_{b1} is H, Cl, Br, OH or -O-C_1-C_{12}alkyl, and R_{b2}, R_{b3} and R_{b5} are, independently of each other H, OH, SH, NH_2, $NHNH_2$, NHOH, NHO-C_1-C_{12}alkyl, -N=CH-N(C_1-C_{12}alkyl)$_2$, -N=CH-N-cycloalkyl, F, Cl, Br, C_1-C_{12}alkyl, hydroxy-C_1-C_{12}alkyl, amino-C_1-C_{12}alkyl, C_1-C_{12}alkoxy, benzyloxy, C_1-C_{12}alkylthio, where the hydroxyl and amino groups are unsubstituted or substituted by a protecting group, phenyl, benzyl, primary amino having from 1 to 20 C atoms or, secondary amino having from 2 to 30 C atoms, R_{b4} is hydrogen, CN or -C≡C-R_{b7}, R_{b6} and R_{b7} are hydrogen or C_1-C_4alkyl.

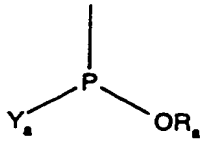
39. A compound according to claim 38, wherein the protecting group for hydroxyl and amino groups is C_1-C_8acyl.

40. A compound according to claim 38, wherein the protecting group for amino groups is -N=CH-N(C_1-C_{12}alkyl)$_2$ or -N=CH-N-cycloalkyl.

41. A compound according to claim 35, wherein R_6 is a phosphorus-containing residue which forms a nucleotide-bridging group of the formula P1 or P2



(P1),

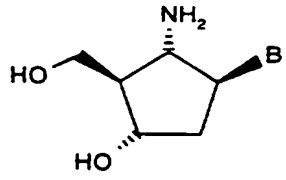


(P2)

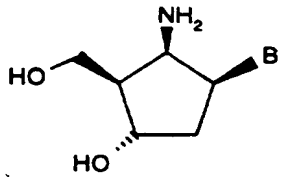
in which Y_a is hydrogen, C_1-C_{12}alkyl, C_6-C_{12}aryl, C_7-C_{20}aralkyl, C_7-C_{20}alkaryl, -OR_b, -SR_b, -NH_2, primary amino, secondary amino, O^-M^+ or S^-M^+; X_a is oxygen or sulphur; R_a is hydrogen, M^+, C_1-C_{12}alkyl, C_2-C_{12}alkenyl or C_6-C_{12}aryl, or the group R_aO- is N-heteroaryl-N-yl having 5 ring members and from 1 to 3 N atoms; R_b is hydrogen, C_1-C_{12}alkyl or C_6-C_{12}-aryl; and M^+ is Na^+, K^+, Li^+, NH_4^+ or is primary, secondary, tertiary or quaternary ammonium; where alkyl, aryl, aralkyl and alkaryl in Y_a, R_a and R_b is unsubstituted or is substituted by alkoxy, alkylthio, halogen, -CN, -NO_2, phenyl, nitrophenyl or halophenyl.

42. A compound according to claim 41, wherein R_a is β-cyanoethyl and Y_a is di(i-propyl)-amino.

43. A compound of the formula IVc' or IVd'



(IVc')

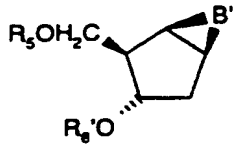


(IVd')

in which B has one of the meanings given above for the residues of the formulae Ia and Ib.

44. A process for preparing a compound of the formula IVc' or IVd' according to claim 43, which comprises reacting

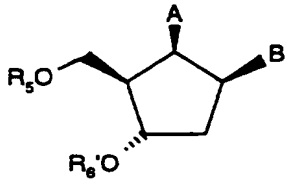
(a) a compound of the formula V



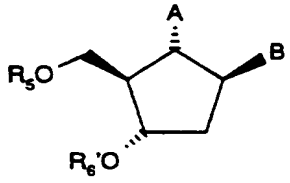
(V)

in which R_5 and R_6' are, independently of each other, a protecting group, and B' is a pyrimidine residue which is bonded via N and O, or

(b) a compound of the formula (VIa) or (VIb),



(VIa)



(VIb)

in which R_5 and R_6' are, independently of each other, a protecting group, B has one of the meanings given above, and A is a customary leaving group, for example a tosylate or a mesylate,

with a metal azide, for example NaN_3, reducing the azido group to the amino group and eliminating the protecting groups.

45. A pharmaceutical preparation which comprises an effective amount of a nucleoside of the formulae IVc' or IVd' according to claim 43 on its own or together with other active compounds, a pharmaceutical excipient material and, if desired, auxiliary substances.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 95/04840

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D473/00 C07H21/00 C07F9/6561 C07F9/6512 C07F9/6558
C07D487/04 C07D471/04 C07D239/54 C07D239/46 C07D403/04
A61K31/52 A61K31/505

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07D C07H C07F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 418 007 (GLAXO GROUP LIMITED) 20 March 1991 see page 6-7 ---	35
A	WO,A,91 01318 (GLAXO GROUP LIMITED) 7 February 1991 see page 6-7; claim 7 ---	35
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A	EP,A,0 267 878 (CIBA-GEIGY AG) 18 May 1988 see claims ---	35

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search
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Date of mailing of the international search report
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Luyten, H

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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A	JOURNAL OF THE CHEMICAL SOCIETY. CHEMICAL COMMUNICATIONS, no. 13, 1 July 1987 pages 1083-1084, KEITH BIGGADIKE ET AL Page 1084: formula 2,3,6 ---	35
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A	JOURNAL OF MEDICINAL CHEMISTRY, vol. 36, no. 14, 9 July 1993 pages 1923-1937, cited in the application see the whole document ---	1
A	TETRAHEDRON, vol. 48, no. 4, 24 January 1992 pages 571-623, ALAN D. BORTHWICK ET AL cited in the application Page 602:formula 235 and 237 ---	35
A	PERSPECTIVES IN MEDICINAL CHEMISTRY, 1 January 1993 pages 275-297, HEINZ E. MOSER cited in the application see the whole document -----	1

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PCT/EP 95/04840

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